VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) ACTIVITY

IN ACID MN-RICH SOIL

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ABSTRACT

Acid soils are known as infertile soils as a results of nutrient deficiency such as P, Ca, Mo or toxicity by Al, Mn. Applying P fertilizer to these soils might not be affordable to farmers; therefore, mycorrhizal inoculation to such soils could be an alternative because these fungi can increase nutrient uptake especially P by associated plants.

Liming is commonly practiced to improve fertility of acid soils since liming can increase soil pH, availability of nutrients such as Ca, P and alleviate toxicity by Al, Mn. Therefore, beneficial effect of liming on mycorrhizal fungi activity might be due to higher soil pH, better status of soil nutrients or alleviation of Al and Mn toxicity.

A glasshouse experiment was conducted to evaluate the effects of soil pH or Ca amendment on vesicular-arbuscular mycorrhizal symbiosis in an acid Wahiawa soil with *Leucaena leucocephala* and *Acacia mangium* as indicator plants.

At target soil solution P concentration of 0.02 mg L^{-1}, *Glomus aggregatum* developed normally at soil pH ranges of 5.0-6.0. However, mycorrhizal effectiveness measured in terms of pinnule P content, nutrient uptake (except Mn), and shoot dry weight attained highest values at pH 6.0 in leucaena and at pH 5.0 in acacia.

In a second study in which I compared the effect of lime with that of gypsum, amendment of soil with gypsum at

0.02 mg P L^{-1} increased VAM colonization. However, VAM colonization was lower if soil was amended with gypsum rather than with lime. In leucaena, higher mycorrhizal effectiveness, nutrient uptake (except Mn), and shoot dry weight were observed in the limed soil than in the gypsum amended soil. However, these values were highest when acacia was grown in the soil amended with 0.32 mg Ca kg^{-1} soil.

The lower VAM colonization and VAM effectiveness observed in leucaena and acacia grown in uninoculated soil probably reflect the inherently low infective propagules in the test soil.

High P in soil caused suppression of mycorrhizal development. This is evidenced by lower VAM colonization in roots of leucaena and acacia grown in the soil with high P than in that with the lower P.

The results of the present study suggest 2 approaches in order to maximize mycorrhizal activity in manganiferous acid soils. Firstly, soil pH must be raised to reduce Mn toxicity if acid tolerant host plants that are sensitve to Mn are to be grown. Secondly, further increase of soil pH to approximately 6.0 is required if acid sensitive host plants are to be grown.

The present study did not succeed to separate the effect of H^{+}, Ca^{+2} and Mn^{+2} on the effectiveness of mycorrhizal symbiosis. Therefore, further research needs to be conducted. Firstly, medium which is inherently low in Mn

such as quartz sand, could be used to distinguish the effect of H^+ from that of Mn^{+2}. Secondly, Zero net charge soil which is low in Ca and Mn such as the Kapaa soil at a depth of 60-90 cm could be used to differentiate the influence of Ca^{+2} addition from that of H^+, and Mn^{+2}.

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INTRODUCTION

Phosphorus deficiency in tropical acid soils is one of the major constraints limiting crop production. This happens because of the high phosphorus fixation capacity of tropical soils which is related to high aluminum (Al), and iron (Fe) contents.

Applying P fertilizer for correcting P deficiency will be relatively expensive since high rates of phosphorus application is needed to supply the P-fixing properties of tropical soils. Besides, the price of P fertilizer might not be affordable to most farmers in tropical countries. Therefore, an inexpensive alternative is needed. Inoculation with appropriate VAM fungi is suggested as one of the relatively low input alternatives (Sanchez and Salinas, 1981).

The most important type of mycorrhizal fungi in agriculture are vesicular-arbuscular mycorrhizal (VAM) fungi that belong to the family of endogonaceae. Vesicular-arbuscular mycorrhizal (VAM) fungi often improve growth of associated plants and this is mainly caused by an increase in nutrient uptake, especially P (Mosse, 1981 and Hayman, 1982).

The effectiveness of VAM symbiosis is governed by the soil environment, plant species and species of endophytes. Among the soil factors, soil pH uniquely influences the

formation and functioning of VAM fungi. Some species of VAM fungi are restricted to acid or alkaline soils while others occur in both alkaline and acid soils (Robson and Abbot, 1989). Thus, inoculation of acid tolerant VAM fungi in acid tropical soils is likely to be effective in overcoming P deficiency. However, if such inocula are not available the use of acid sensitive VAM inocula may be inevitable. If so, liming is necessary to adjust soil pH favorable to VAM inocula. The availability and cost of lime is also concern encountered in tropical countries. Nevertheless, lime application is inevitable in acid soils when acid sensitive VAM fungi are to be utilized.

When lime is applied to soils, hydroxyl ions produced cause soil pH to increase. Hydroxyl ions also precipitate Al and Fe so that toxicity of these elements is eliminated and phosphorus which was initially fixed by Al and Fe becomes more available. Calcium in the soil solution is also increased as a result of the dissociation of lime which releases Ca^{+2}. Lime also increases oxidation reactions of Mn that produces unavailable Mn. Therefore, lime application decreases Mn toxicity. Thus, the beneficial effects of lime on VAM fungi symbiosis could be caused by more proper pH, increased availability of nutrients such as P and Ca and/or reduced toxicity of Al, Fe, Mn. Aziz and Habte (1989) found that liming simulatedly eroded soils increased root colonization by *Glomus aggregatum* and mycorrhizal shoot dry

weight. However, they did not elucidate whether the improved VAM effectiveness they observed was caused by the more suitable pH, higher availability of nutrients or alleviation of toxic nutrients such as Mn. The objective of the present study were:

1. Evaluate the influence of soil pH on the development of VAM activity in *Leucaena leucocephala* and *Acacia mangium* in the Wahiawa soil at low pH.
2. Determine whether calcium or soil pH influences the development of VAM activity in *Leucaena leucocephala* and *Acacia mangium* in the Wahiawa soil at low pH.

CHAPTER 1

LITERATURE REVIEW

The Importance of Vesicular-Arbuscular Mycorrhizae (VAM)

The roots of most healthy plants are able to establish a mutual association with particular fungi. Such association is called mycorrhizae (Jackson and Mason, 1984). Due to their ability to produce structures so called vesicles and arbuscules in the cortical tissue of roots, these fungi are named vesicular-arbuscular mycorrhizae (VAM) (Powell and Bagyaraj, 1984). Mosse (1981) defined arbuscules as intracellular, haustoria-like structures while vesicles as sack-like structures that develop in root system. Vesicular-arbuscular mycorrhizal symbiosis is found in most plant families, however, Hirrel *et al.* (1978) reported that this symbiosis was rare and even absent in the roots of plant ascribed to families of cruciferae, chenopodiaceae, carryophyllaceae, and cyperaceae.

Vesicular-arbuscular mycorrhizal fungi are found to be beneficial in agriculture because VAM fungi can increase nutriet uptake, especially P (Mosse, 1981; Hayman, 1982; Aziz and Habte, 1987; Habte *et al.* 1987; Powell, 1975; Sanni, 1976; Yost and Fox, 1979; Lu and Miller, 1989). Higher P uptake by mycorrhizal plants is mainly due to

exploration of larger soil volume (Rhodes and Gerdemann, 1975; Bolan, 1991). In addition, hyphae of VAM fungi is very effective in improving soil aggregation (Sutton and Sheppard, 1976), VAM fungi increase plant growth in soil having water stress (Allen and Boosalis, 1983; Graham and Syvertsen, 1984; Huang *et al.*, 1985; Michelsen and Rosendahl, 1990; Sympson and Daft, 1990), reduce the susceptibility to soil-borne pathogens like nemathodes, phythophthora (Mosse, 1981; Hayman, 1982; Gianinazzi-Pearson and Gianinazzi, 1983).

Acid Soils and Their Problems

Acid soils, Ultisols and Oxisols, in the tropics occupy the largest area of land (Sanchez and Salinas, 1981). High average temperatures and rainfalls cause intensive weathering; consequently, the soil are weathered fast and yielding Ultisols and Oxisols that are characterized by low pH (Fanning and Fanning, 1989).

Acid soils are known as low productive soils because of nutrient deficiency such as phosphorus, molybdenum, calcium, magnesium and by toxicity of aluminum, manganese, or hydrogen ion (Marschner, 1991; Fox *et al.*, 1991).

Soil Acidity and VAM Fungi Distribution

Field survey (Porter *et al.*, 1987a) revealed that there was a correlation between soil pH and the distribution of VAM fungi. The distribution of *Acaulospora laevis* was restricted to soils with pH of 4.5-4.9 but that of *Glomus sp.* was found only in soils having pH more than 6.4 in Western Australia soils. Spores of *Acaulospora laevis* and *Glomus monosporum* were recovered from Western Australia soils of pH 4.8 whilst *Glomus sp.* (WUM 2) and *Glomus sp.* (WUM 3) were found in Western Australia soils having pH of 7.5 (Porter *et al.*, 1987b). Nicolson and Schenk (1979) collected spores of *A. laevis* from soils with pH of 4-4.5 in central florida. *Glomus monosporum* which naturally occurred in Western Australia soils with pH of 4.8 was adaptable to pH 6.8 in the same soil (Porter *et al.*, 1987b). This indicates that *G. monosporum* can occur in both acid and alkaline soils. Spores of *A. laevis* were also found to greatly dominate acid fields of pH 4.3 to 4.8 while the spore of *G. mosseae* was found in more alkaline fields in Oregon (Young *et al.*, 1985). Wang *et al* (1985) revealed that only at pH of 4.5 did fine endophytes infect root of oat whilst at pH 5.5-7.5 coarse endophytes were found to be dominant.

Soil pH and VAM Fungi Activity

Spore germination of VAM fungi, in general, may be influenced more by soil microorganisms, the physical and chemical environments than by the presence or absence of host or non-host plant roots (Hetrick, 1984). Spore germination of *A. laevis* was depressed at pH 7.4 but that of *Glomus sp.* (WUM 3) was stimulated (Porter *et al.*, 1987b). Green *et al.* (1976) also found that different VAM species responded differently to soil pH with regard to spore germination. They revealed that spore germination of *Gigaspora coralloidae* was higher at pH lower than 6.0 while that of *G. mosseae* was higher at pH higher than 6.0. Increasing pH beyond 5 decreased spore germination of *A. laevis* (Hepper, 1984) but pH 7.0-7.4 was found to be optimum for spore germination of *Glomus epigaeus* (Daniels and Trappe, 1980). However, it is not clear which component of soil pH (H^+ ion *per se*, Al, Mn) depressed spore germination. Porter *et al.* (1987b) and Green *et al.* (1976) showed that the depression of spore germination was due to H^+ ion. On the other hand, Wang *et al.* (1985) found that the formation of mycorrhizae in Oats by *Glomus caledonium* was decreased with increasing the concentration of Al and Mn in solution applied to acid washed sand at pH 4.5.

In general, effect of pH on hyphal growth paralelled effects on spore germination. Higher hyphal growth of *A.*

laevis was associated with pH 4.6 than with pH 7.6; however, *Glomus sp* (WUM 3) responded conversely (Porter *et al.*, 1987b). A beneficial effect of liming on hyphal growth of *Gigaspora margarita* was reported by Siqueira *et al.* (1984). Thus, different species of endophyte demand particular pH ranges in order to achieve optimum hyphal growth.

The factors which stimulate or inhibit root colonization by VAM fungi probably also stimulate or inhibit sporulation since these two phenomena are often closely related (Hayman, 1970; Daft and Nicolson, 1972). Soil pH influences root colonization by VAM fungi in the same way as it influences spore germination and hyphal growth (Robson and Abbot, 1989). Maximum root colonization of corn by *G. mosseae* was attained when soil pH was adjusted to 6.1 and soil was fertilized with 240 ppm P in the form of superphosphate (Siqueira *et al.*, 1984). A contributive effect of lime on root colonization of *Leucaena leucocephala* by *G. mosseae* was also recorded by Huang *et al.* (1983); Hayman and Tavares (1985); Davies *et al.* (1983). It indicates that *G. mosseae* seems to favorably colonize roots of associated plants at neutral pH. Besides, soil pH of the origin of VAM fungi is likely to affect an extent of colonization. *Glomus mosseae* isolated from soil with pH of 6.8 colonized roots of *L. leucocephala* significantly higher when acidic soil (pH 5.2) was limed to pH 7.4 (Huang *et al.* 1983). Colonization of alfalfa by VAM fungi, which was

collected from soil with a pH range of 7.2-7.7, was linearly increased when an acidic soil (pH 5.3) was amended with lime. An increase in root colonization, however, does not always coincide with an increase in dry matter yield. For example, *G. fasciculatum* colonized roots of strawberry equally high at pH 4 and 7 but shoot dry matter yield at pH 7 was significantly higher compared to that at pH 4 (Hayman and Tavares, 1985). Huang *et al.* (1983) also found that *Leucaena leucocephala* with high root colonization by *G. fasciculatum* to a comparable extent (>70%) produced lower dry matter yield at pH 5.2 than pH 5.7.

Inoculation of soil fertilized with 25 ppm P with *G. margarita* at pH 4.5 increased dry matter yield of strawberry. At pH higher than 4.5, inoculation with the same endophyte did not increase dry matter yield (Yawney *et al.*, 1982). Inoculation with *Glomus sp.* significantly increased shoot dry weight of oil palm grown in acid soils (pH 5.1) (Blal *et al.*, 1990). Guzman-Plazola (1988) noted a significant yield increase of Leucaena grown in acid soils (pH 5.3) when inoculated with *Glomus intraradices*. Liming soil to reach certain soil pH significantly increased dry matter yield of sweetgum grown in acid soils inoculated with *G. mosseae* (Davis *et al.*, 1983). Liming was negatively correlated with root colonization by native VAM fungi but was positively correlated with colonization by VAM fungus isolated from soil of pH 7.2-7.4 (Kucey and Diab, 1984). In

acid soils, the effectiveness of *G. aggregatum* seems to be favored by addition of lime (Aziz and Habte, 1989; Aziz and Habte, 1990; Habte and Aziz, 1991). These previous studies show that effect of lime on VAM symbiosis in acid soils is likely to be determined by the response of VAM fungi to acidity. Acid adapted endophytes will not respond or be affected by liming. In contrast, acid-sensitive endophytes will benefit from liming. In addition, soil fertility i.e. soil phosphorus noticed in the previous studies varied and this might also lead to different responses of VAM fungi to soil acidity. Phosphorus is believed to detrimentally influence mycorrhizal effectiveness (Same *et al.*, 1983; Kucey and Diab, 1984; Siqueira *et al.*, 1984; Habte *et al.*, 1987; Sainz and Arines, 1988; Braunberger *et al.*, 1991). Therefore, influence of soil pH on mycorrhizal effectiveness needs to be examined under fixed level of soil solution phosphorus.

Liming acid soils is aimed at increasing soil pH. However, the availability of some nutrients like P, Ca is also increased while the availability of Mn or Al is reduced. Yawney *et al.* (1982) reported that liming an acid soil from pH 4.5 to 6.5 significantly increased extractable Ca of a Bladen sandy loam soil as well as root colonization by *G. gigantea*. Dry matter yield of sweetgum was significantly increased when soil pH was increased from pH 5.1 to 7.6 and soil was inoculated with *G. mosseae* (Davis *et*

al., 1983). Thus, an increase in soil pH was accompanied by an increase in extractable Ca and a decrease in extractable soil Mn. A similar finding was also reported by Siqueira *et al* (1984). Literatures cited indicate that better VAM activity in limed soils could be due to more appropriate pH, higher soil Ca, less toxicity of Al or Mn. However, these previous studies did not elucidate which component of soil acidity potentially stimulate or harm VAM fungi. Aluminum (Al) and manganese (Mn) have already been known to detrimentally affect mycorrhizal formation (Wang *et al*. 1985). Hence, influence of Ca in soil solution on VAM formation and effectiveness needs to be tested.

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CHAPTER 2

GENERAL MATERIALS AND METHODS

Soil Used and Preparation

Wahiawa soil was used in this experiment. The soil is classified as Rhodic Eutrustox, clayey, Kaolinitic, Isohyperthermic. The soil was collected from the Poamoho experimental site at a depth of 7.5 cm to 15 cm. This soil is known to be Mn-rich with an original pH of 4.9. Soil samples were air-dried and crushed to pass through a 4-mm sieve. Each plastic pot (15 x 15 cm) was filled with 2 kg of air-dried soil.

Indicator Plants Used

Leucaena leucocephala and *Acacia mangium* were used in this study. The former is known to be sensitive to acidity (Hutton, 1981; Olvera *et al.*, 1982; Balasundaran *et al.*, 1988; Halinda, 1988) and the latter is tolerant (Glover and Heuveldop, 1985; Halinda, 1988). Homogenous and healthy seeds were selected, scarified and sterilized to break dormancy and to obtain seedlings free from pathogens. Seeds of leucaena were immersed in concentrated sulfuric acid for 20 minutes and then washed with sterilized water 6 times.

Acacia seeds were soaked in boiling water and left in it until the water cooled down.

Soil pH Determination

Twenty milliliter of deionized water or 0.01 M $CaCl_2$ was added to ten gram of dry soil sample. This mixture was stirred with glass rod for 2 minutes and 15 minutes afterwards, the pH of samples was determined with a Fisher pH meter model 805 MP.

Mycorrhizal Inoculum Used

Crude inoculum of *Glomus aggregatum* was used to inoculate the soils. The crude inoculum consisted of infected root, hyphae, spores and sand. This inoculum was obtained from Dr. M. Habte, Department of Agronomy and Soil Science, the University of Hawaii. Inoculation was achieved by thoroughly mixing 30 g of the inoculum with each 2 kg of dry soil. The inoculum was applied one day before planting.

Basal Fertilization

All experimental soils received a blanket nutrient solution. It was composed of: KCl, $MgSO_4.7\ H_2O$, $CuSO_4.\ 5H_2O$, H_3BO_3, $Na_2MoO_4\ .2H_2O$, $ZnSO_4.\ 7H_2O$ and $Mg(NO_3)_2.\ 6H_2O$ at the rate

of 100, 50, 5, 10, 0.5, 10, and 69.2 mg of K, Mg, Cu, B, Mo, Zn, and N respectively per Kg of soil (Aziz and Habte, 1987). The amount of deionized water used to dissolve the nutrients per pot was adjusted to meet 60% of water holding capacity. The blanket nutrients were applied at planting.

Experimental Design

The experiment was undertaken in the University of Hawaii Agronomy and Soil Science glasshouse under natural light (21° 51′ N and 156° 22′ W). Treatments were arranged in randomize block design with 3 replicates per treatment. Two seedlings were grown per pot and five days later were thinned to one plant. Pots were watered with deionized water as needed to maintain approximately 60% of water holding capacity. Data were analyzed using the SAS procedure (SAS Institute Inc., 1991).

Plant Height Measurement

Plant height was measured by means of a meter stick from the soil surface to the youngest shoot.

Vesicular-Arbuscular Mycorrhizal Development

The procedure developed by Habte *et al.* (1987) was employed to monitor the development of VAM effectiveness. The third pinnule from the base of the youngest fully expanded leaf of leucaena or acacia was sampled every 5 days beginning from 15 days after planting. Pinnule samples were ashed for 3 hours in a muffle furnace at 500°C before P was determined (Riley and Murphy, 1962).

Shoot Dry Weight and Root Colonization

The above ground plant parts were oven-dried at 70°C until constant weight was obtained and dry weight was recorded. Roots were washed and stained using the method of Kormanik *et al.* (1980). The stained roots were observed under a dissecting microscope and the degree of VAM colonization was assessed using the grid line intersect method (Giovanetti and Mosse, 1980).

Plant Tissue Analysis

Oven-dried above ground plant parts were ground in a stainless steel Wiley mill. Twenty five milligram of the ground tissue was ashed for about 4 hours in a muffle furnace at 500°C. The ash was dissolved in 10 mL of 1 N HCl

and digested to dryness on a hot plate. Afterwards, 10 mL of 1 N HNO_3 was added. This solution was analyzed for Ca, Mg, Cu, Zn, Mn with an atomic absorption spectrophotometer. Phosphorus was determined by the molybdate blue method (Murphy and Riley, 1962).

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CHAPTER 3

EFFECT OF SOIL pH ON VESICULAR ARBUSCULAR MYCORRHIZAE

INTRODUCTION

Soil acidity is one of the soil chemical properties that might influence the distribution and/or persistence of beneficial microorganisms including vesicular-arbuscular mycorrhizal (VAM) fungi. Porter *et al*. (1987a and 1987b) have found that VAM fungi predominantly existed in acid soils, germinate better and produce better hyphal growth in acid soils than in alkaline soils. Some species of VAM fungi functioned effectively in acid soils and others in neutral to alkaline soils (Davis *et al*., 1983; Huang *et al*., 1983; Abbot and Robson, 1985; Hayman and Tavares, 1985). These studies; however, were not carried out under optimal soil conditions especially for soil P. Phosphorus was applied at different amounts without knowing how much P was available. The concentration of P in soil solution could be different from one soil to another even though the same amount of P is applied because soils have different P fixing capacities. The initial soil solution P required for optimum mycorrhizal symbiosis with highly dependent plant is 0.02 mg L^{-1} (Manjunath and Habte, 1990). Therefore, mycorrhizal response at a particular soil pH observed in the previous studies might not be maximum.

The objective of the present study was to determine the soil pH which is favorable for the formation and function of mycorrhizal association between *G. aggregatum* and *L. leucocephala* or *A. mangium*.

MATERIALS AND METHODS

To establish different soil pH levels, an incubation study was carried out. Different concentrations of sulfuric acid or calcium hydroxide were added to 250 g air-dry soil. Soil was incubated at 28°C and at approximately 60% of water holding capacity. Soil pH was monitored every week until constant pH values were attained. The target pH's were 4.3, 5.0, 5.5, and 6.0. Based on the incubation study , 0.025 M H_2SO_4 was required to obtain pH 4.3. While concentrations of lime needed to establish pH 5.0, 5.5, and 6.0 were 0.0079 mol, 0.024 mol, and 0.0402 mol $Ca(OH)_2$ kg^{-1} soil, respectively. Three weeks were required to attain pH equilibrium. On the basis of the incubation study, lime or sulfuric acid corresponding to the target pH's was added to 2 kg air dry soil 3 weeks before P application. Sulfuric acid was applied as a solution while lime was thoroughly mixed with soil.

Two levels of soil solution P namely 0.02 mg L^{-1} and 0.8 mg L^{-1} were used in the study. Soil solution P was established using the procedure described by Fox and

Kamprath (1970). Target P levels were established three weeks after the soil had been amended with lime or sulfuric acid. Habte and Manjunath (1987), Manjunath and Habte (1990) found that optimum mycorrhizal symbiosis was associated with 0.02 mg P L^{-1} soil solution. Soil solution P of 0.8 mg L^{-1} was found to be adequate for non-mycorrhizal plants (Habte and Manjunath, 1987). Potassium phosphate monobasic at the amounts to attain target P levels were applied 15 days before planting.

Mycorrhizal inoculation was obtained by mixing 30 g of crude inoculum of *G. aggregatum* with 2 kg air dry soil one day before planting.

L. leucocephala (Lam) de Wit cv. K8 and *A. mangium* NFTA 276a were grown under natural light in the glasshouse of the University of Hawaii Agronomy and Soil Science (21° 51'N and 156°22'W) from June 13 to July 28, 1991. Two seedlings were transplanted and five days later were thinned to one plant per pot. The average temperature during the experiment was 31.2°C.

The parameters measured to evaluate mycorrhizal response to soil pH were soil chemical properties before planting and after harvesting, plant height, VAM development, shoot dry weight, VAM colonization, and chemical composition of plants after harvest. Data collected were analyzed by using the SAS procedure (SAS Institute INC., 1991).

RESULTS

Manganese in the soil solution declined with increasing soil pH and its availability was negligible at pH 5.0 or higher. Magnesium in the soil solution or that extracted with ammonium acetate was not affected by soil pH. However, extractable Ca as well as Ca in the soil solution were significantly influenced by soil pH (Table 3.1).

Soil Chemical Properties Before Planting

Table 3.1. Effect of soil pH on soil chemical properties before planting.

Treatment Soil pH (1:2 H_2O)	Soil pH (1:2 0.01 M $CaCl_2$)	Nutrients in soil solution			NH_4OAC exct. elements	
		Mn	Mg	Ca	Mg	Ca
		-----mg L^{-1}------			---mg kg^{-1}---	
4.3	4.08	16.6	2.7	7.1	34	260
5.0	4.47	0.15	3.5	19.4	40	560
5.5	5.07	ND	3.7	40.4	36	1020
6.0	5.37	ND	3.9	63.8	24	1120

ND=Not detected.

Soil pH and Mn in the soil solution relatively did not change markedly after harvest in soil with a target P level of 0.02 mg L^{-1} (Table 3.2). At the higher target P level, soil pH was increased. In soil with a target level of pH 4.3 and 0.8 mg P L^{-1}, soil pH increased from 4.3 to 4.7 and Mn dropped to 1.9-2.1 mg L^{-1}.

Soil Chemical Analysis After Harvest

Table 3.2. Effect of soil pH on soil chemical properties after harvest.

Target pH	Soil pH (1:2 H_2O) Leucaena	Acacia	Soil Mn (mg L^{-1}) Leucaena	Acacia
	---------- 0.02 mg P L^{-1} ----------			
4.3	4.36 d	4.37 d	15.1	13.2
5.0	4.98 c	4.93 c	ND	ND
5.5	5.35 b	5.21 b	ND	ND
6.0	5.67 a	5.69 a	ND	ND
	---------- 0.8 mg P L^{-1} ----------			
4.3	4.76 d	4.75 d	1.9	2.1
5.0	5.31 c	5.24 c	ND	ND
5.5	5.53 b	5.55 b	ND	ND
6.0	5.88 a	5.94 a	ND	ND

+VAM=inoculated with G. aggregatum; -VAM=uninoculated; ND=not detected. Figures in the same column under different target P levels with the same letter are not significantly different at the 5% probability level by the LSD test.

Vesicular-Arbuscular Mycorhhizal (VAM) Colonization

There was a significant interaction effect of VAM inoculation and soil pH on VAM colonization in leucaena and acacia grown in soil with target P concentration of 0.02 mg L^{-1} but not in those grown in soil with P concentration of 0.8 mg L^{-1} (Figs. 3.1 and 3.2).

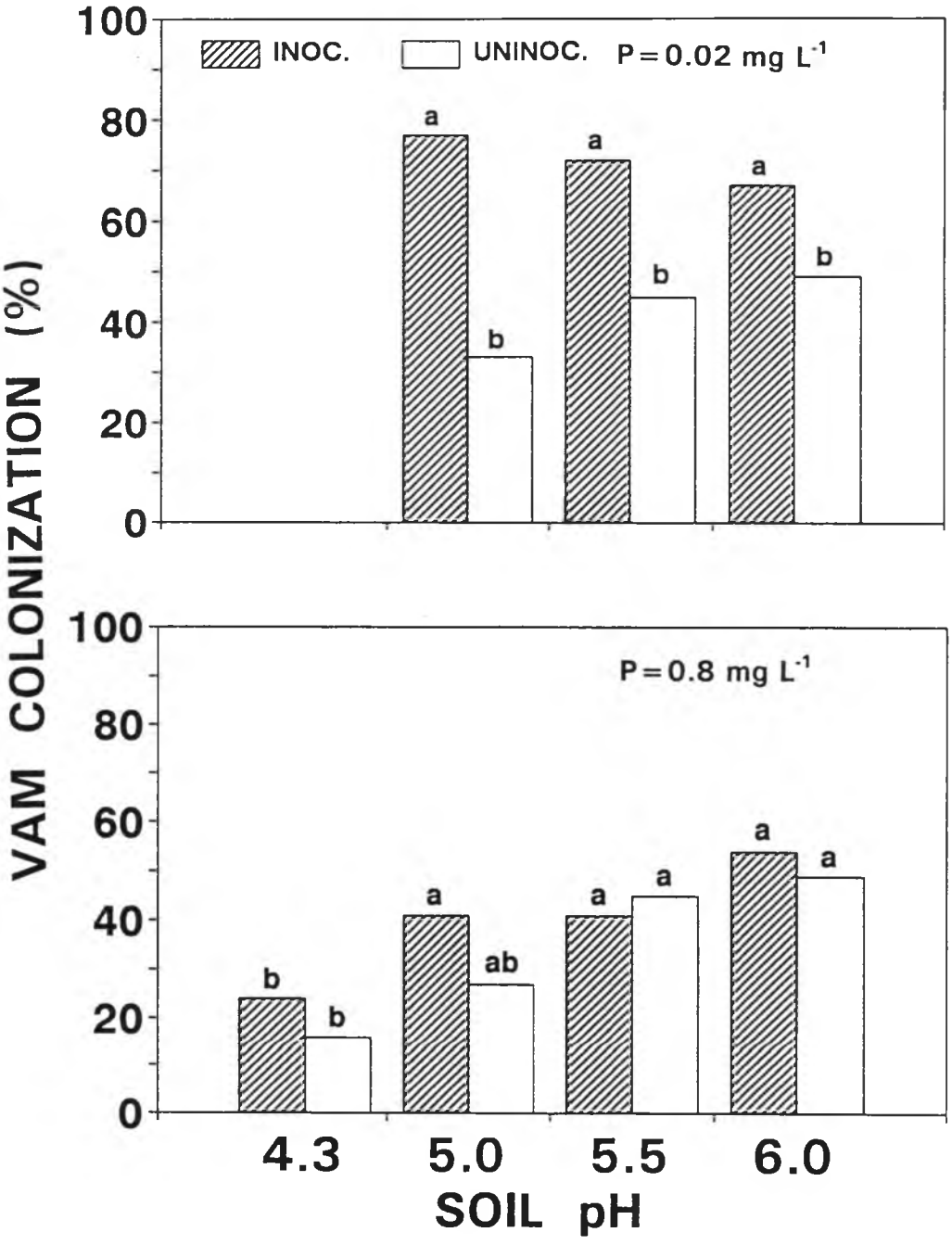

Fig.3.1. The influence of VAM inoculation, soil pH and P concentration on VAM colonization of leucaena roots. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

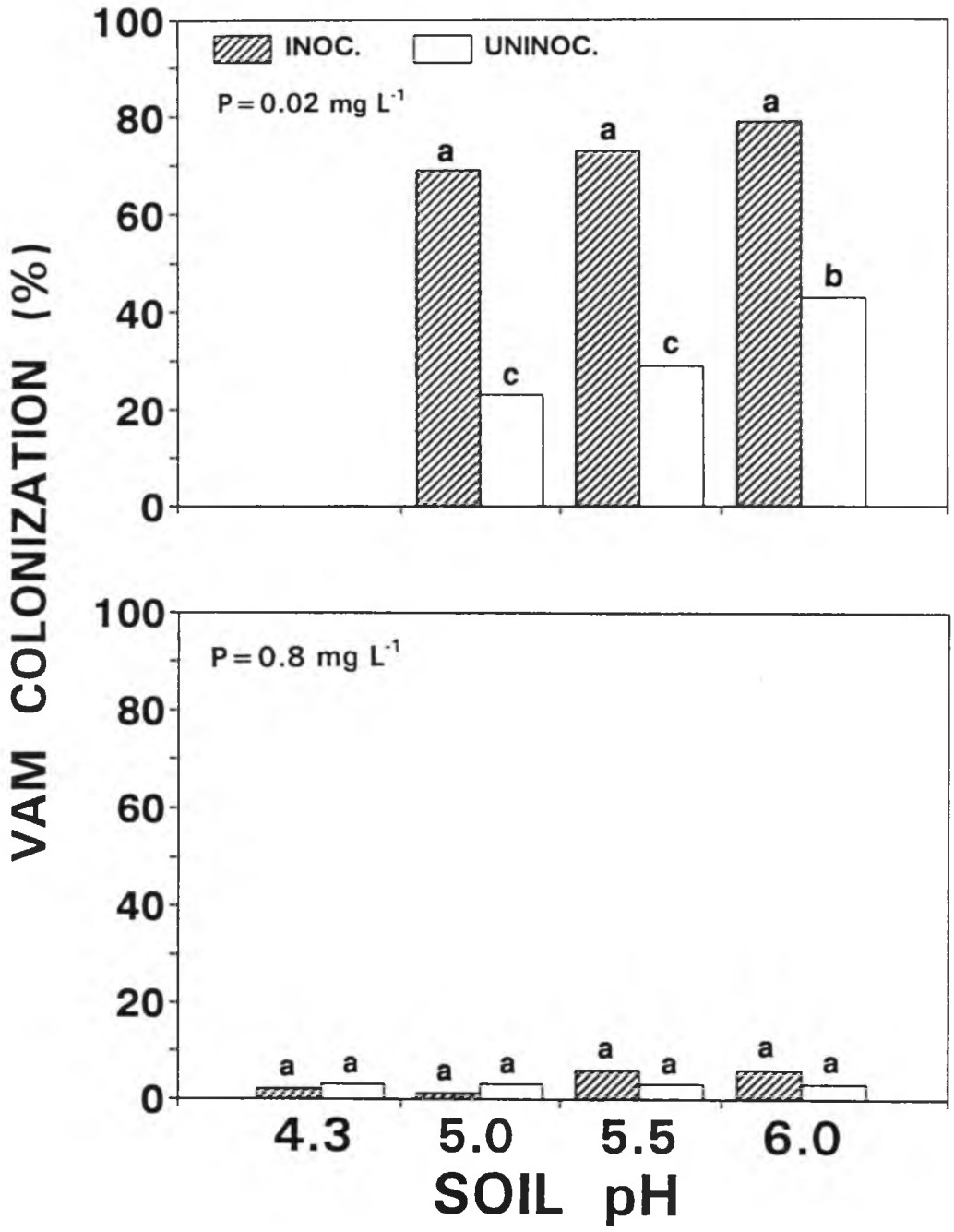

Fig.3.2. The influence of VAM inoculation, soil pH and P concentration on VAM colonization of acacia roots. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

At pH 4.3, neither roots of leucaena nor those of acacia were colonized by VAM fungi at low P level but VAM colonization was detected in the soil with 0.8 mg P L^{-1}. High Mn and or H^+ may have been responsible for the absence of colonization in the low P soil.

In soil with 0.02 mg P L^{-1}, inoculation with *G. aggregatum* induced a significant increase of VAM colonization in leucaena and acacia when soil pH was raised to 5.0. No further increase of VAM colonization by *G. aggregatum* was found at soil pH higher than 5.0.

Development of Vesicular-Arbuscular Mycorrhizal (VAM) Activity

There was no mycorrhizal activity in leucaena and acacia grown in soil with pH 4.3 except in soil with target P concentration of 0.8 mg L^{-1}. In soil with target P level of 0.02 mg L^{-1}, mycorrhizal activity in leucaena grown in inoculated soil started to be higher from that grown in uninoculated soil at 25 , 20, and 15 days after planting (DAP) if soil pH's were 5.0, 5.5, and 6.0, respectively (Fig. 3.3). When target soil P was 0.8 mg L^{-1}, mycorrhizal activity in leucaena grown in inoculated soil started to be higher from that grown in uninoculated one at 30, 25, and 20 DAP at soil pH's of 5.0, 5.5, and 6.0, respectively (Fig. 3.4). Mycorrihzal development was delayed by 5 days if soil

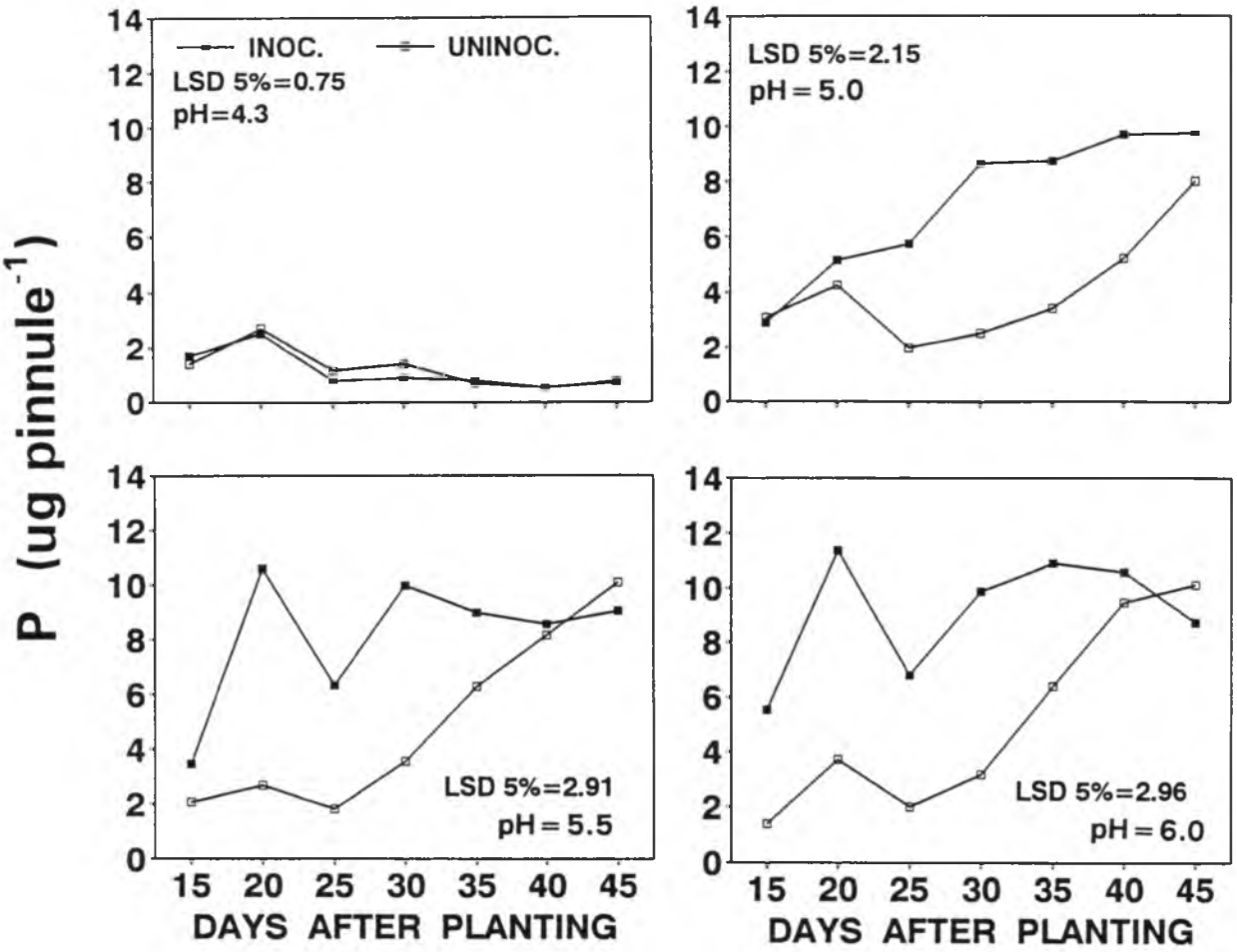

Fig.3.3. The influence of VAM inoculation and soil pH on the development of mycorrhizal effectiveness in leucaena grown in soil with 0.02 mg P L^{-1}.

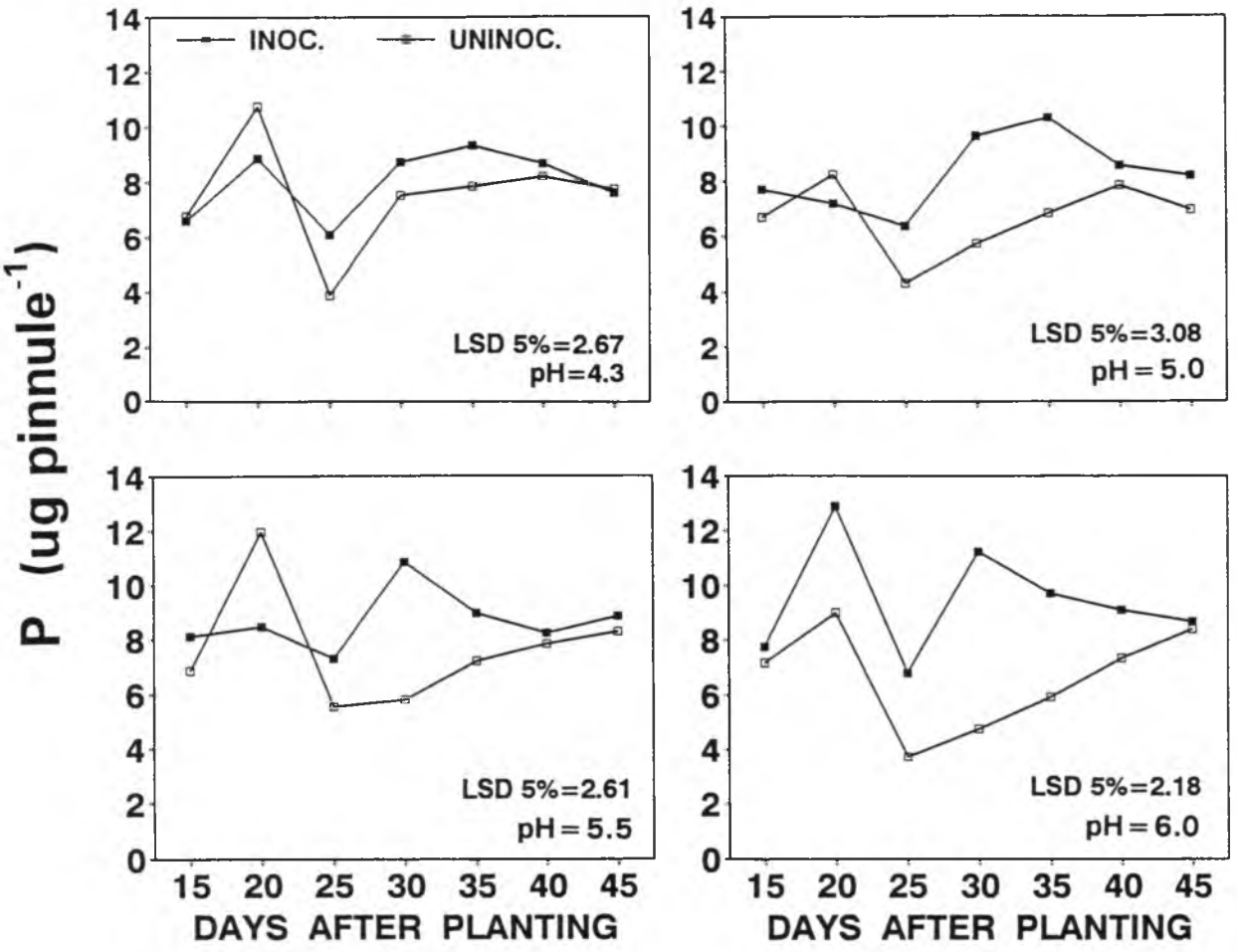

Fig.3.4. The influence of VAM inoculation and soil pH on the development of mycorrhizal effectiveness in leucaena grown in soil with 0.8 mg P L^{-1}.

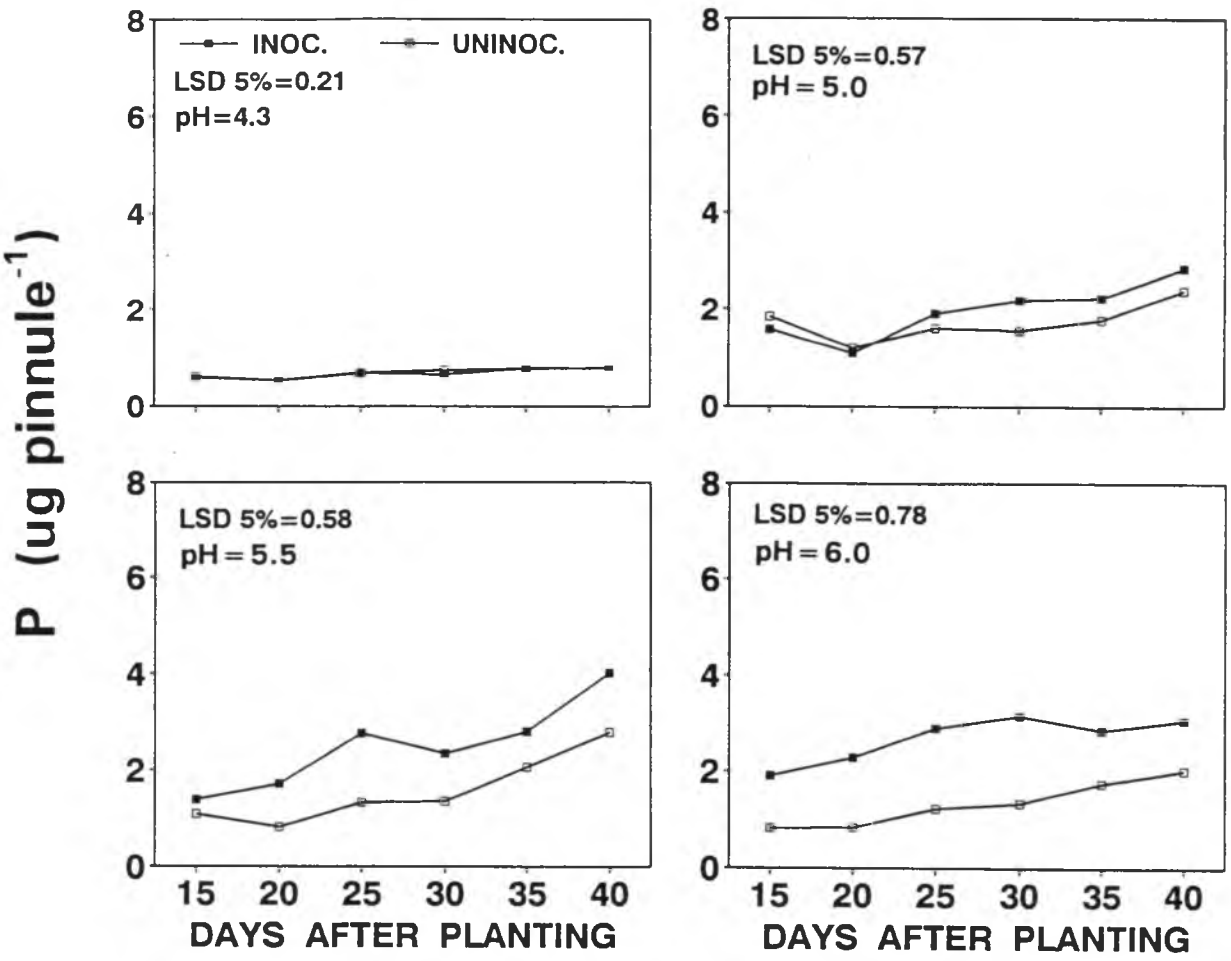

Fig.3.5. The influence of VAM inoculation and soil pH on the development of mycorrhizal effectiveness in acacia grown in soil with 0.02 mg P L^{-1}.

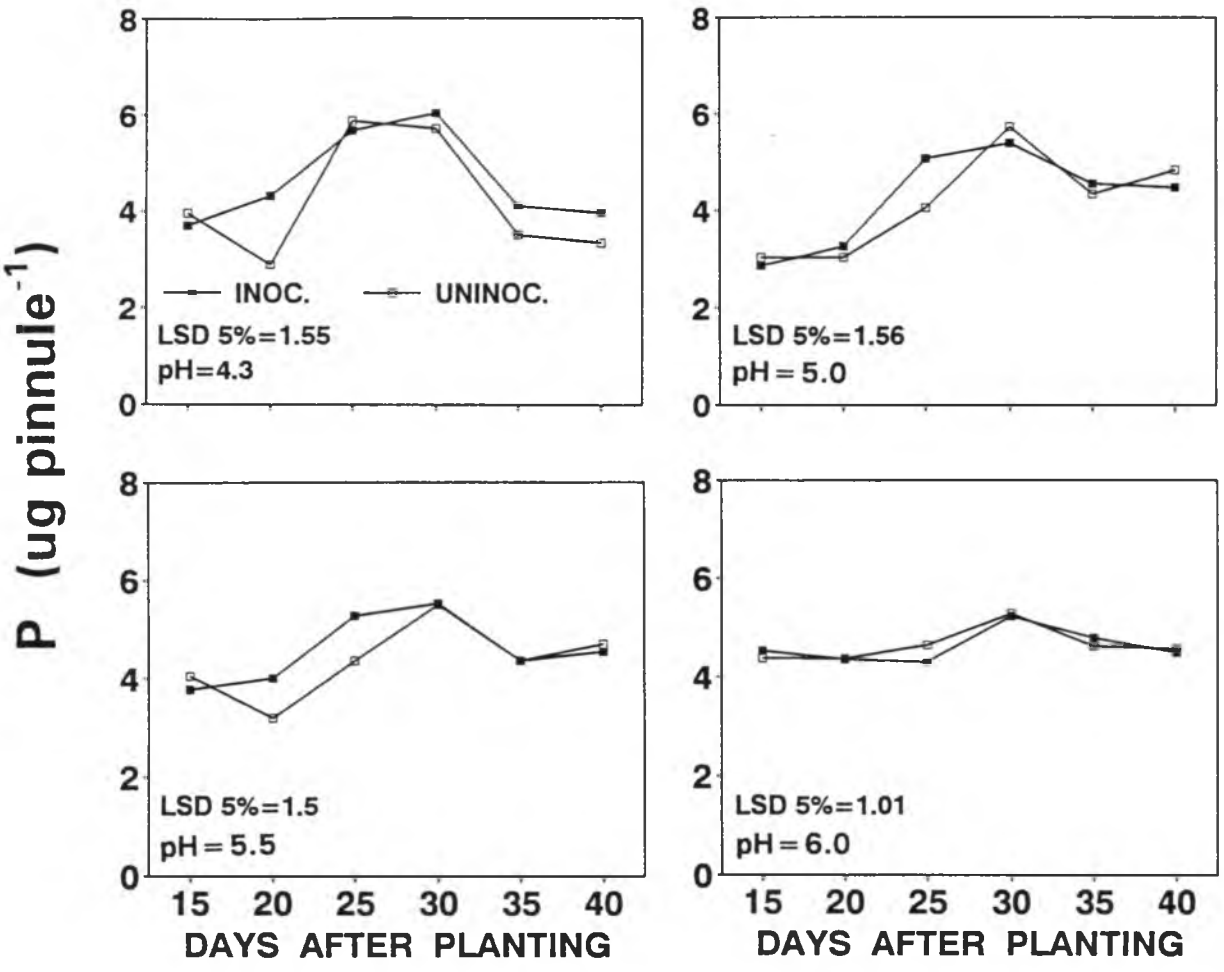

Fig.3.6. The influence of VAM inoculation and soil pH on the development of mycorrhizal effectiveness in acacia grown in soil with 0.8 mg P L^{-1}.

P was increased from 0.02 mg L^{-1} to 0.8 mg L^{-1}.

Acacia grown in soil with target P level of 0.02 mg L^{-1} started showing higher mycorrhizal activity in inoculated soil than in uninoculated soil beginning 30, 20 and 15 DAP with soil pH 5.0, 5.5, and 6.0, respectively (Fig. 3.5). At higher soil P level, the development of mycorrhizal activity in uninoculated soil was similar to that in the uninoculated soil (Fig. 3.6).

Shoot Dry Weight

Shoot dry weight of leucaena in soil with 0.02 mg L^{-1} increased as soil pH was increased from 4.3 to 5.5 and further increase was achieved when soil pH was increased to 6.0 and inoculated with *G. aggregatum (Fig.* 3.7). Maximum shoot dry weight of leucaena grown in soil with P concentration of 0.8 mg L^{-1} was obtained at soil pH of 6.0 and inoculated with *G. aggregatum*. Shoot dry weight of leucaena grown in inoculated soil with pH 6.0 was 146% higher than that of leucaena grown in uninoculated soil with target P level of 0.02 mg L^{-1} but only 43% higher in soil with target P level of 0.8 mg L^{-1}.

Shoot dry weight of acacia was significantly influenced by soil pH and mycorrhizal inoculation only at soil P concentration of 0.02 mg L^{-1} (Fig. 3.8). At this P concentration, inoculation with *G. aggregatum* significantly

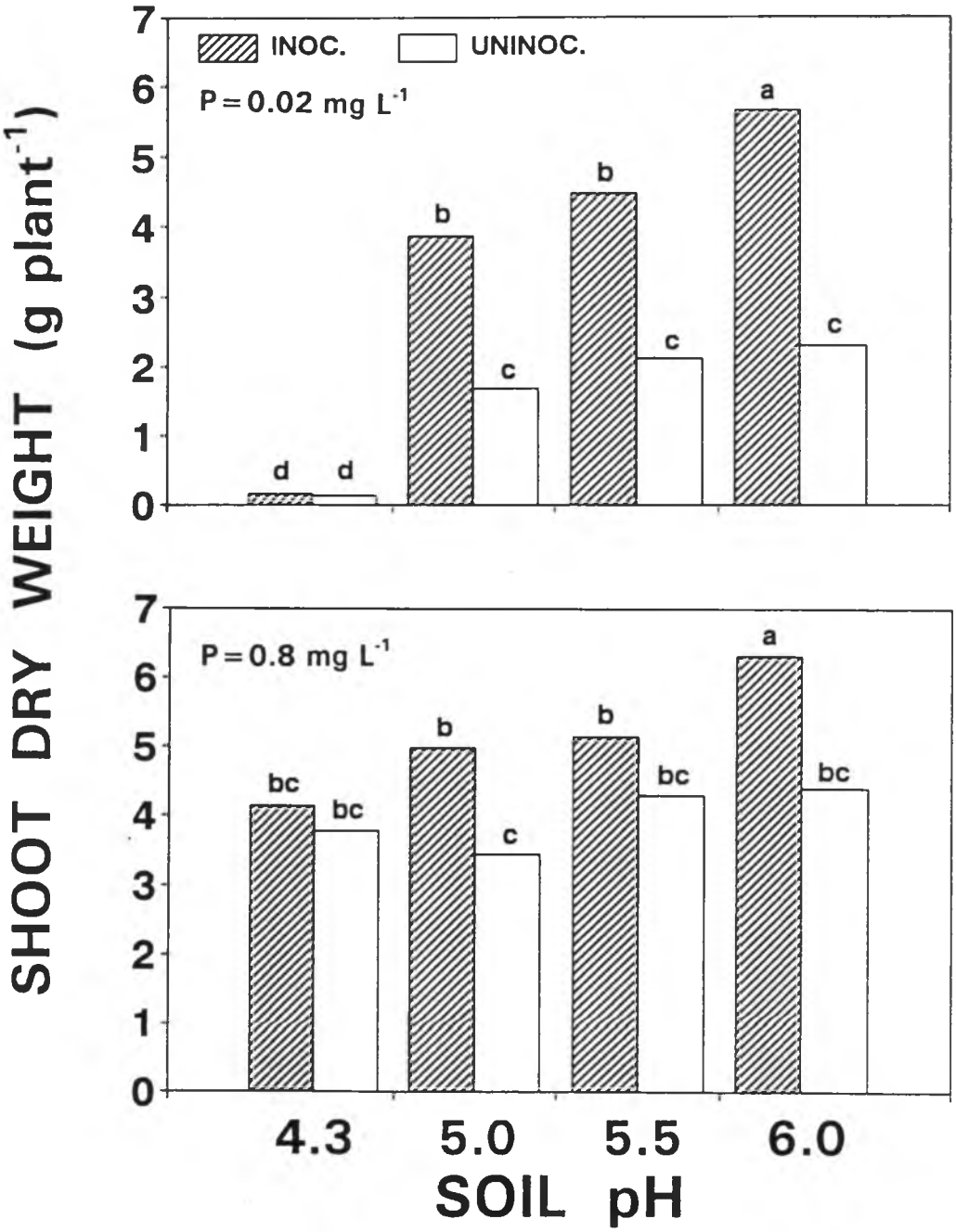

Fig.3.7. The influence of VAM inoculation, soil pH and P concentration on shoot dry weight of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

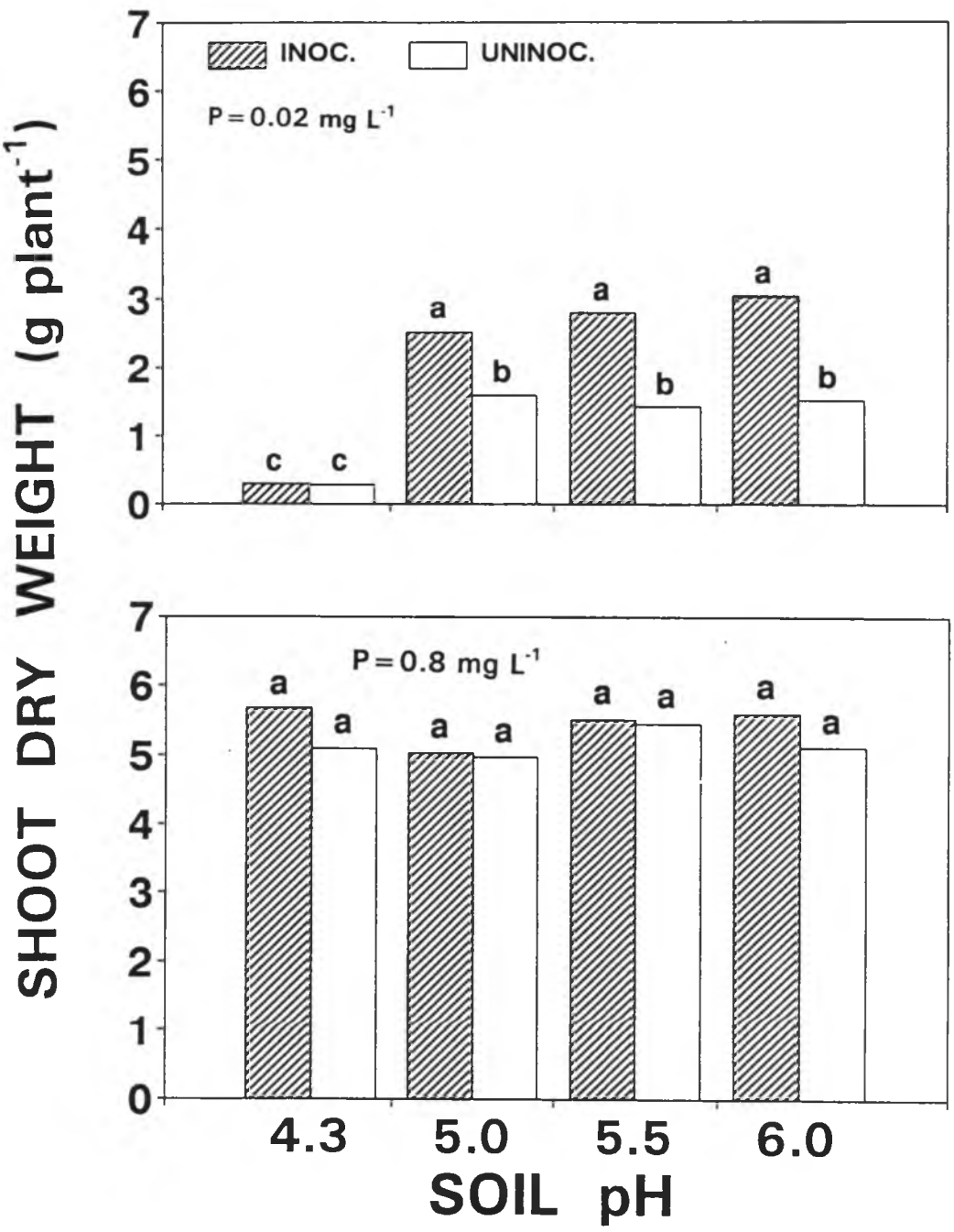

Fig.3.8. The influence of VAM inoculation, soil pH and P concentration on shoot dry weight of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

increased shoot dry weight of acacia when soil pH was raised from 4.3 to 5.0. Further increase of soil pH did not increase shoot dry weight. Shoot dry weight of acacia in the inoculated soil at 0.02 mg P L^{-1} was about 50% lower than that of acacia in uninoculated soil with 0.8 mg P L^{-1}.

Root Dry Weight

Root dry weight of leucaena and acacia grown in soil with P concentrations of 0.02 and 0.8 mg L^{-1} increased when soil pH was increased to 5.0 (Figs. 3.9, and 3.10). Further increase was noted when soil was also inoculated with *G. aggregatum*. Root dry weight did not increase as pH increased above 5.0 and with mycorrhizal inoculation. At P concentration of 0.8 mg L^{-1}, root dry weight of leucaena increased with mycorrhizal inoculation at pH 5.0 but above pH 5.0 further increase was not noted. Root dry weight of acacia was not influenced by soil pH and mycorrhizal inoculation at higher P concentration.

Plant Height

Figures 3.11 and 3.12 show that soil pH and mycorrhizal inoculation had no effect on height of leucaena and acacia at 12 days after planting (DAP) irrespective of soil P concentrations tested. Leucaena and acacia may be supported

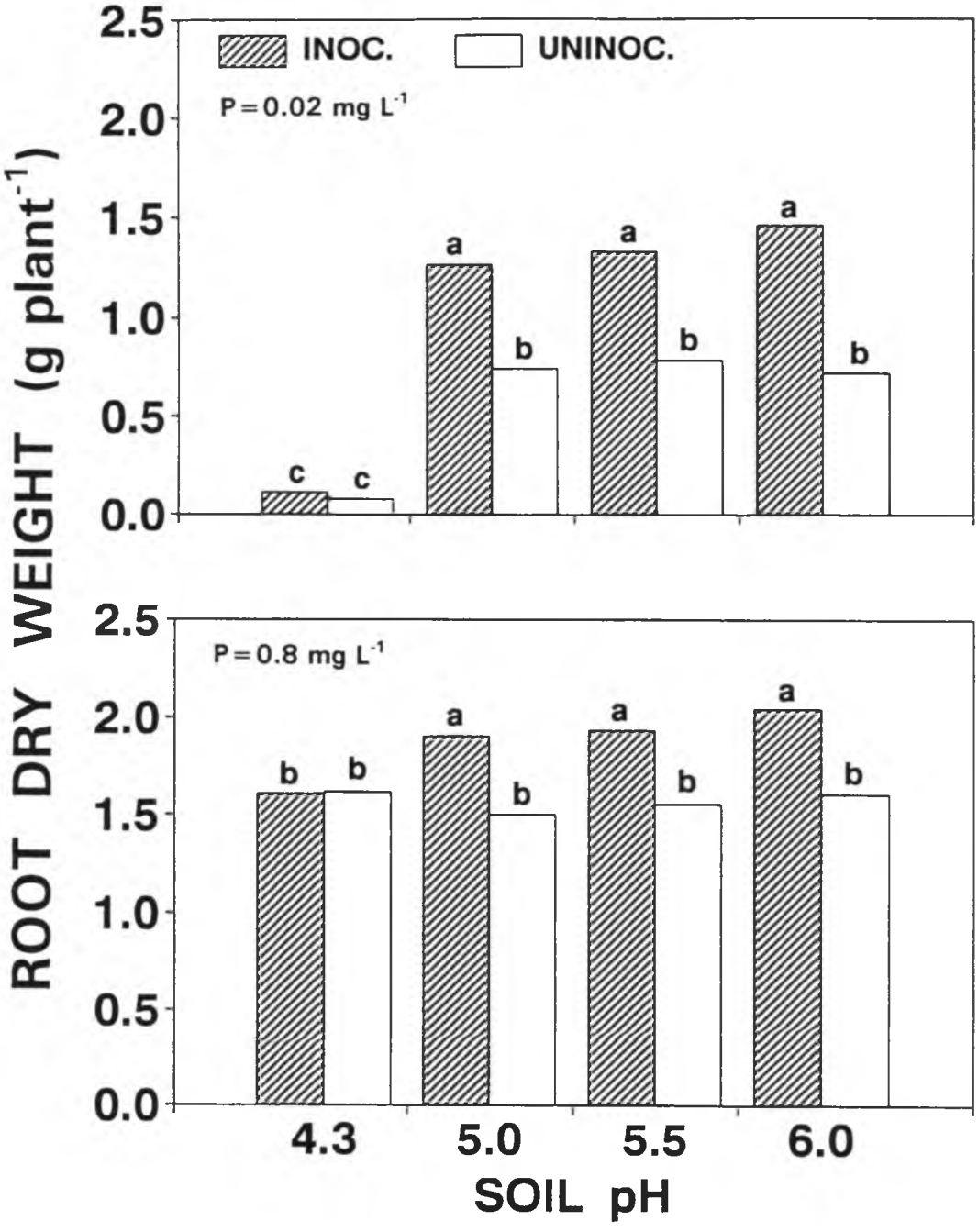

Fig.3.9. The influence of VAM inoculation, soil pH and P concentration on root dry weight of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

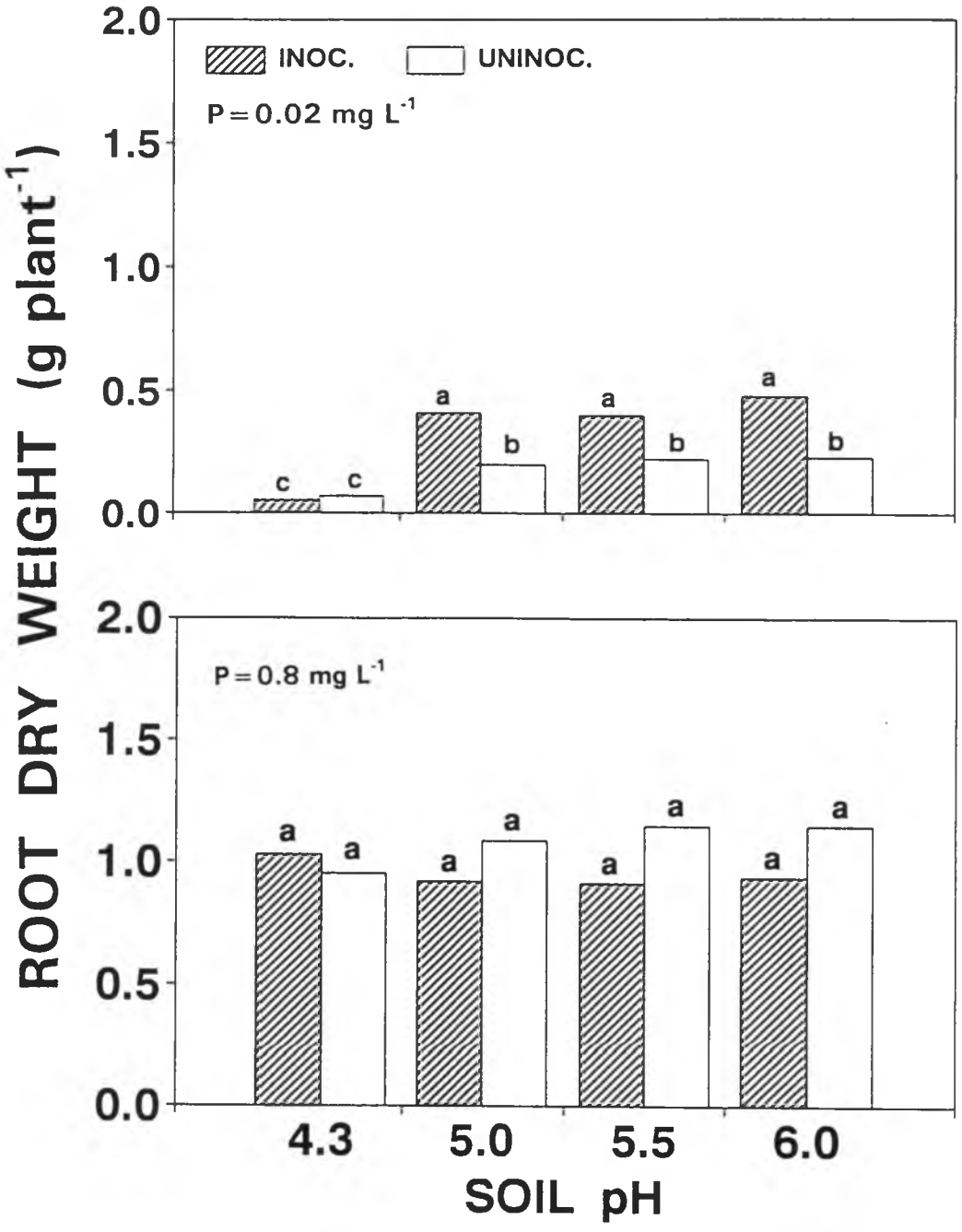

Fig.3.10. The influence of VAM inoculation, soil pH and P concentration on root dry weight of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

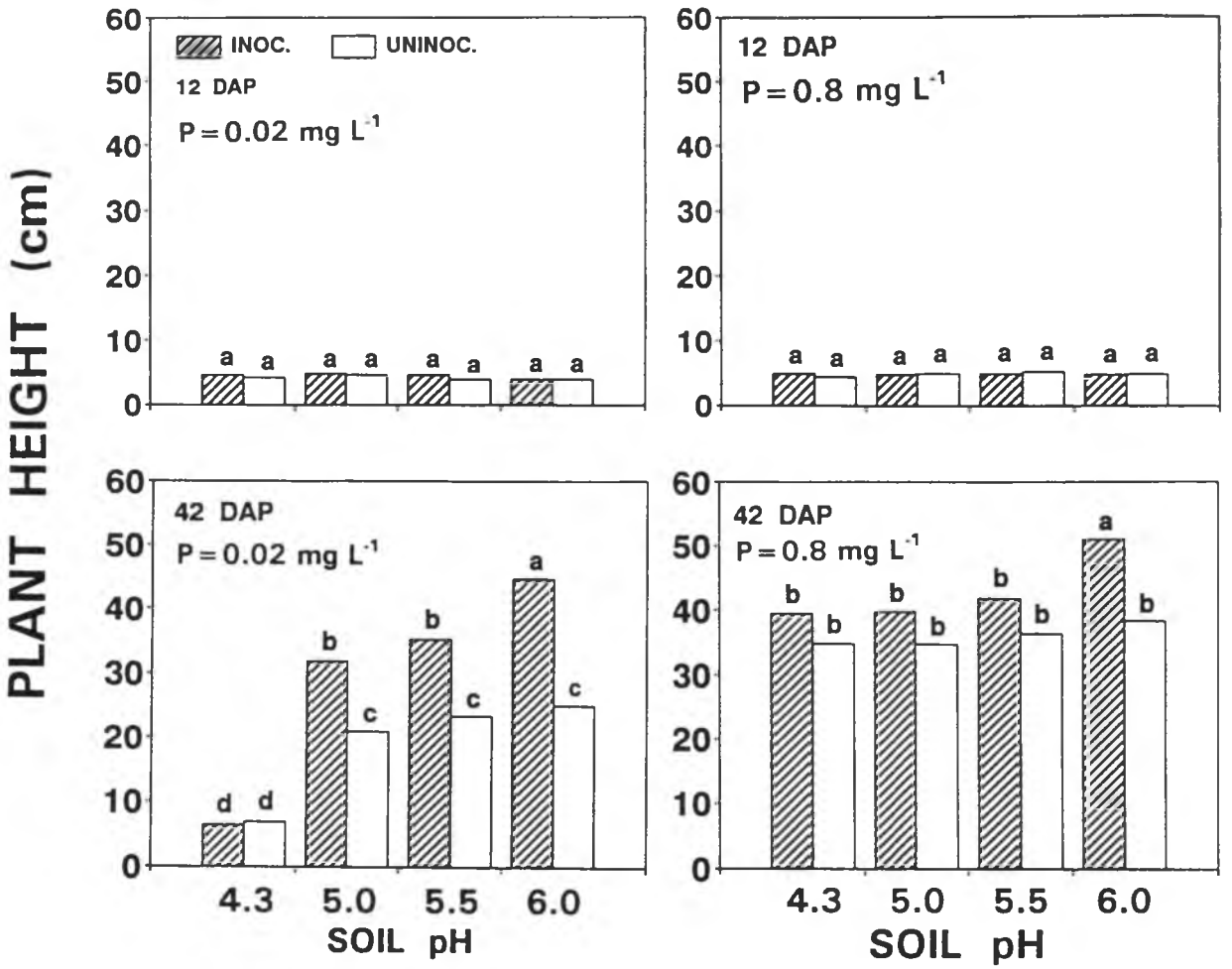

Fig.3.11. The influence of VAM inoculation, soil pH and P concentration on plant height of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

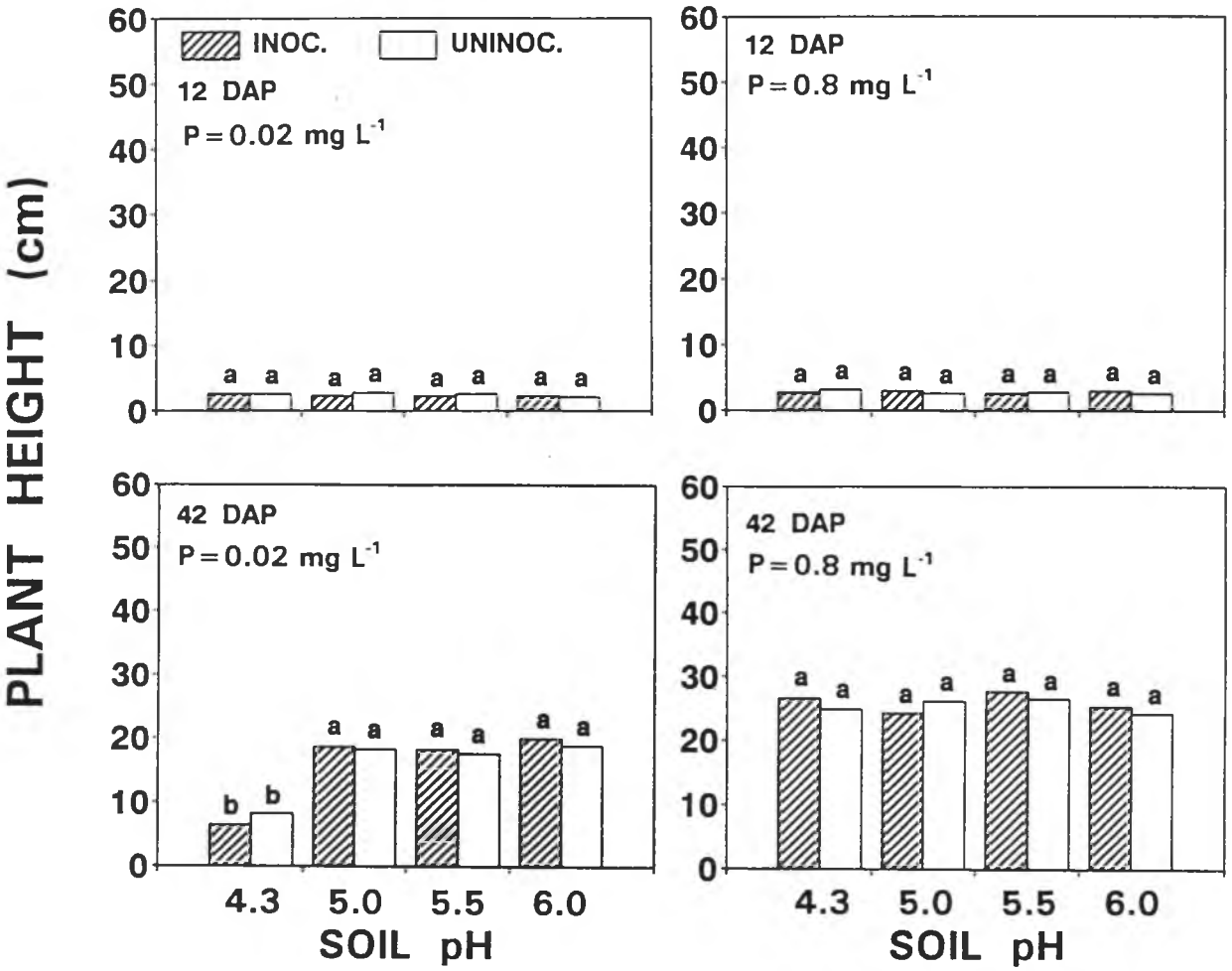

Fig.3.12. The influence of VAM inoculation, soil pH and P concentration on plant height of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

sufficiently through food reserve in their seeds until 12 DAP. At 42 DAP, growth of leucaena was maximum if soil was inoculated with *G. aggregatum* and pH was raised to 6.0 regardless of soil P concentration. In soil with 0.02 mg P L^{-1}, only soil pH influenced height of acacia at 42 DAP, but plant height was not higher after pH 5.0 (Fig. 3.12).

Chemical Composition of Plants

Lambert *et al.* (1979) found that better growth of mycorrhizal plants caused a reduction in tissue concentration of Cu and Zn. As a result, there was no significant difference in chemical composition between mycorrhizal and non-mycorrhizal plants (Lambert *et al.*, 1979; Abbot and Robson, 1985b; Bagyaraj *et al.*, 1989). Therefore, chemical composition of plants in the current study is presented in terms of total element content of shoot per plant. However, Mn was expressed in terms of mass per unit mass of dry matter. This was done to determine the concentration of Mn in plants associated with toxicity.

Inoculation of soil with *G. aggregatum* increased shoot Cu of leucaena with an increase of soil pH at both soil P concentrations (Fig. 3.13). However shoot Cu content of leucaena at pH 6.0 was not significantly different from shoot Cu at pH 5.5 regardless of soil P levels tested. Copper content of acacia was increased by mycorrhizal

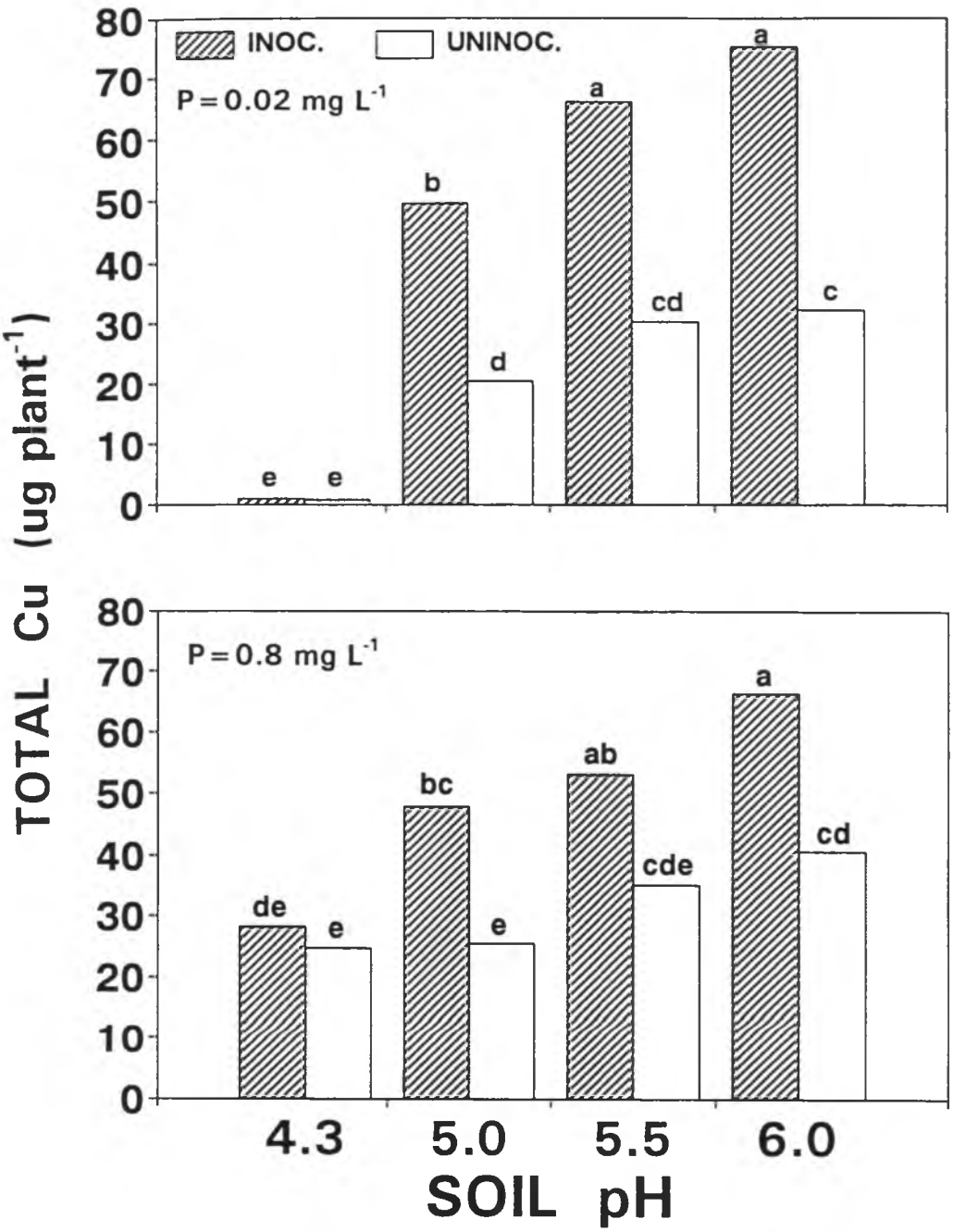

Fig.3.13. The influence of VAM inoculation, soil pH and P concentration on total shoot Cu content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

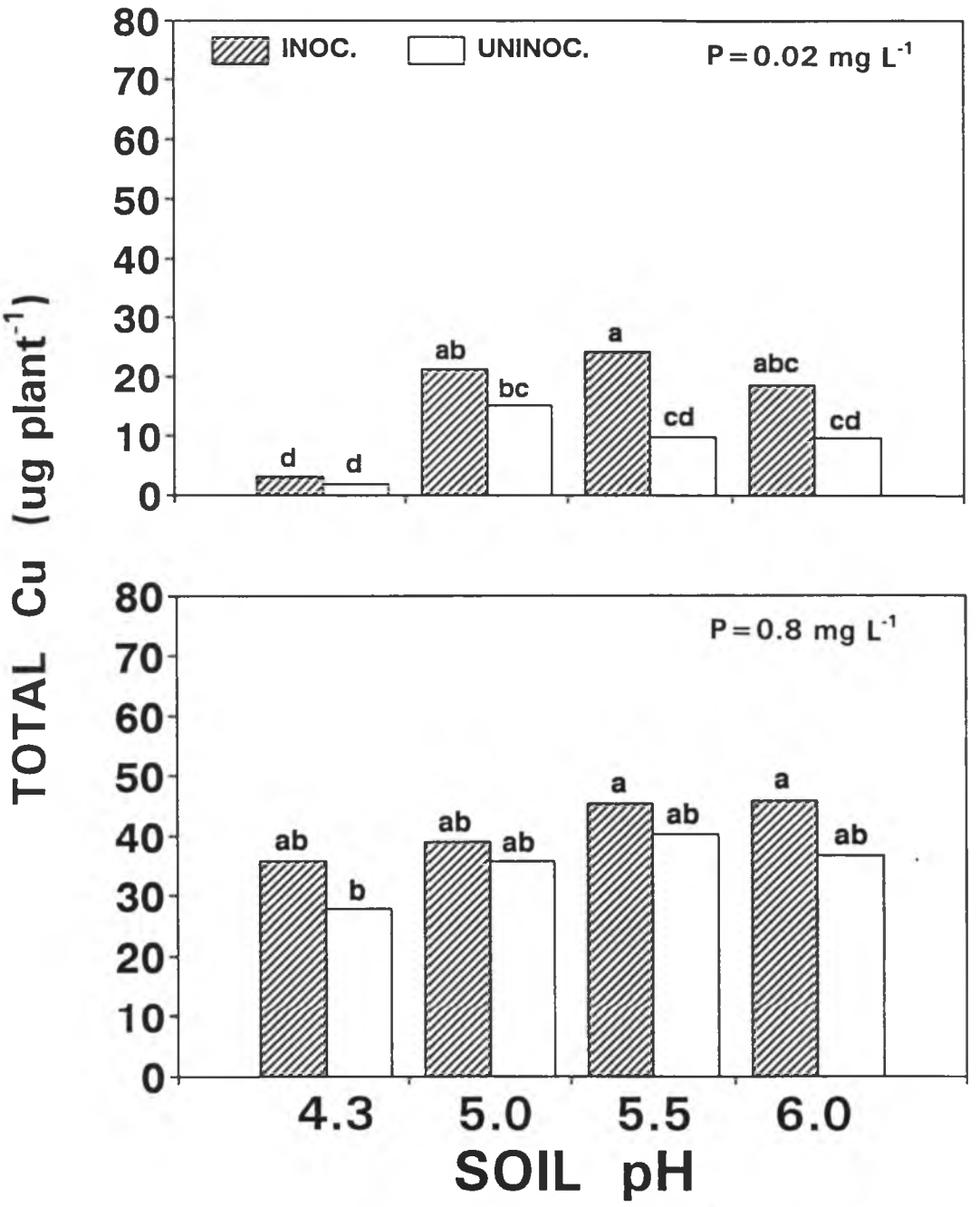

Fig.3.14. The influence of VAM inoculation, soil pH and P concentration on total shoot Cu content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

inoculation only at P concentration of 0.02 mg L^{-1} with soil pH higher than 4.3 (Fig. 3.14). Acacia grown in inoculated soil with an initial P concentration of 0.02 mg L^{-1} had similar contents of shoot Cu at pH 5.0, 5.5, and 6.0.

There was a higher shoot Zn content in leucaena grown in inoculated soil than that grown in the uninoculated soil when pH was increased to 5.0 at both soil P concentrations of 0.02 and 0.8 mg L^{-1}. Further increase of soil pH irrespective of soil P concentrations did not increase shoot Zn content in leucaena (Fig. 3.15). Figure 3.16 depicts an effect of soil pH and mycorrhizal inoculation on shoot Zn of acacia grown in soil with target P concentrations of 0.02 mg L^{-1} and 0.8 mg L^{-1}. At lower P, increasing soil pH from 4.3 to 5.0 or 5.5 led to higher shoot Zn content of acacia in the inoculated soil but not in the uninoculated one. If soil pH was raised to 6.0 shoot Zn content declined and shoot Zn content of acacia in inoculated and uninoculated soil became similar. At P concentration of 0.8 mg P L^{-1} soil with higher P, mycorrhizal inoculation did not enhance shoot Zn content of acacia, but a significant decrease in shoot Zn content was noted at pH 6.0.

Shoot Mn of leucaena or acacia was mainly influenced by soil pH (Figs. 3.17, and 3.18). Highest shoot Mn was observed when leucaena or acacia was grown in soil at pH 4.3 at either P concentrations tested. Pronounced reduction of shoot Mn occurred when soil pH was increased to 5.0.

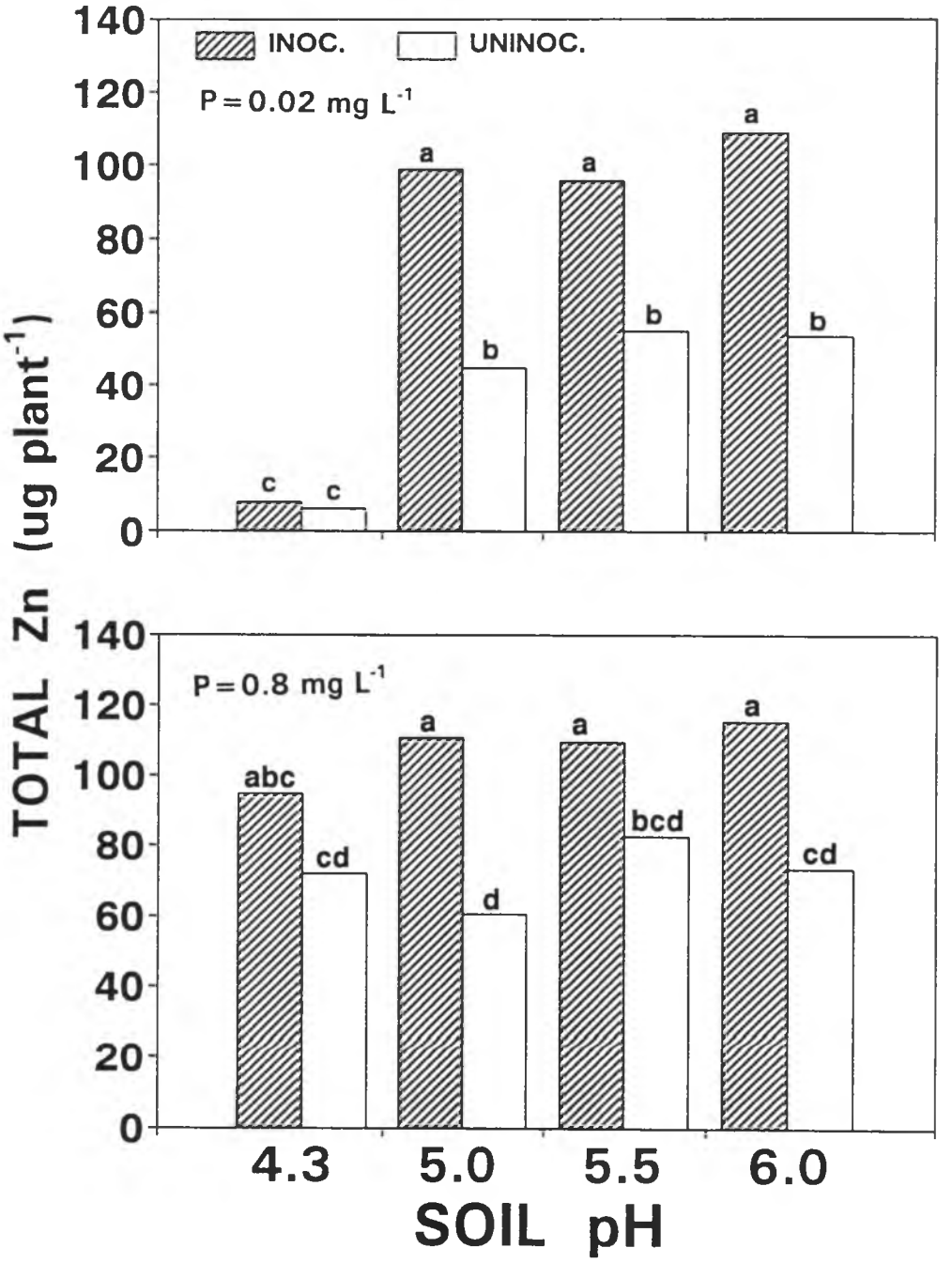

Fig.3.15. The influence of VAM inoculation, soil pH and P concentration on total shoot Zn content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

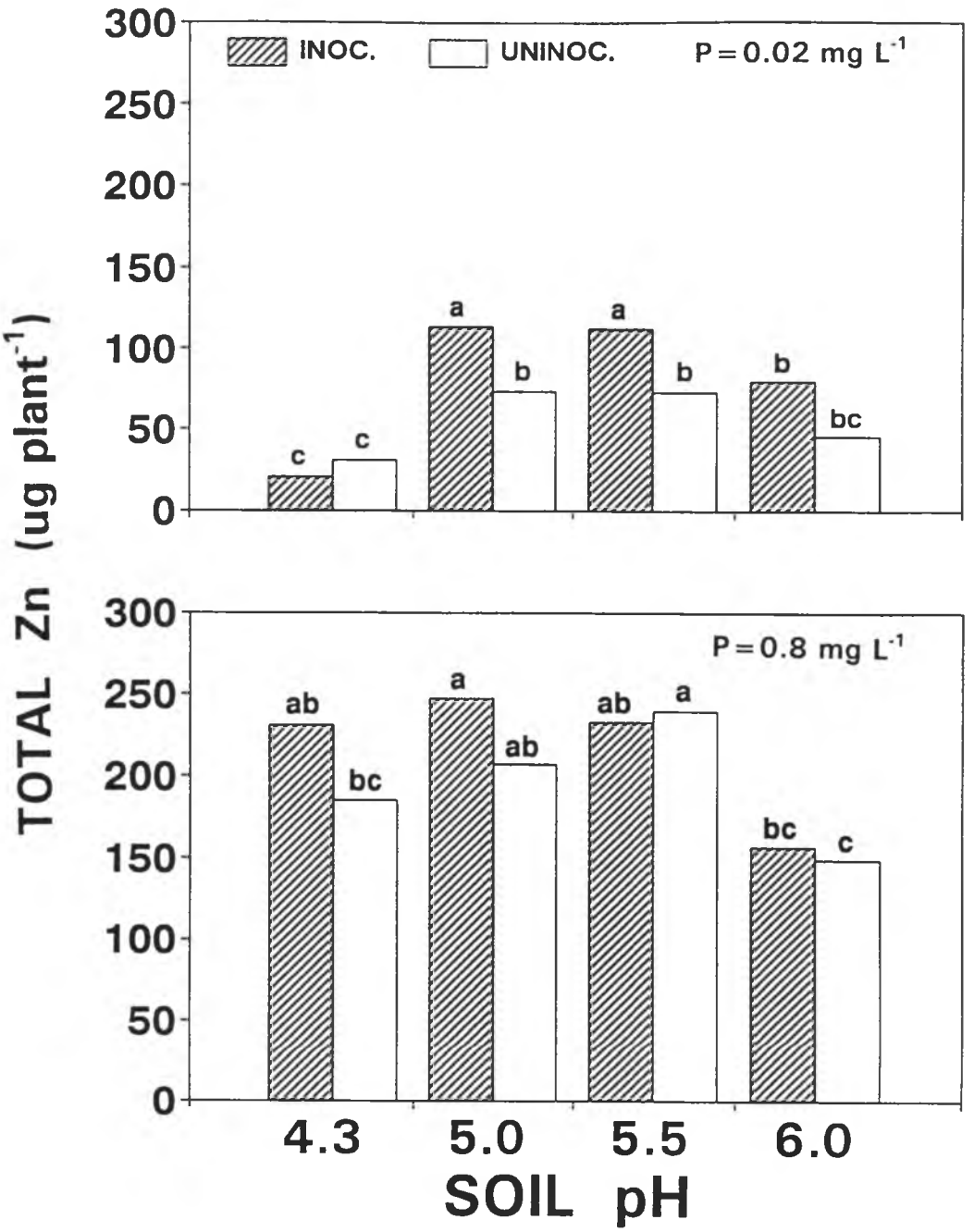

Fig.3.16. The influence of VAM inoculation, soil pH and P concentration on total shoot Zn content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

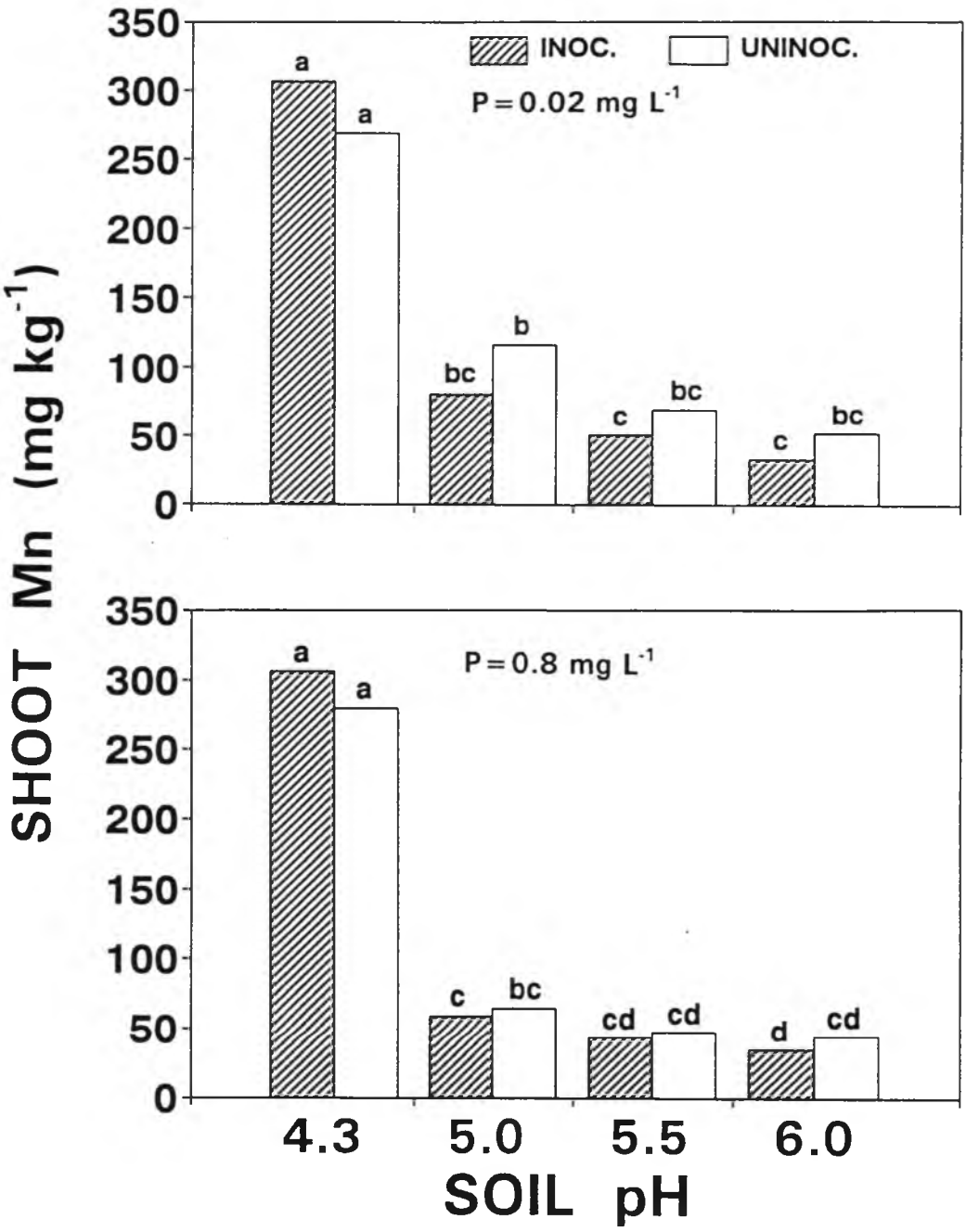

Fig.3.17. The influence of VAM inoculation, soil pH and P concentration on shoot Mn content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

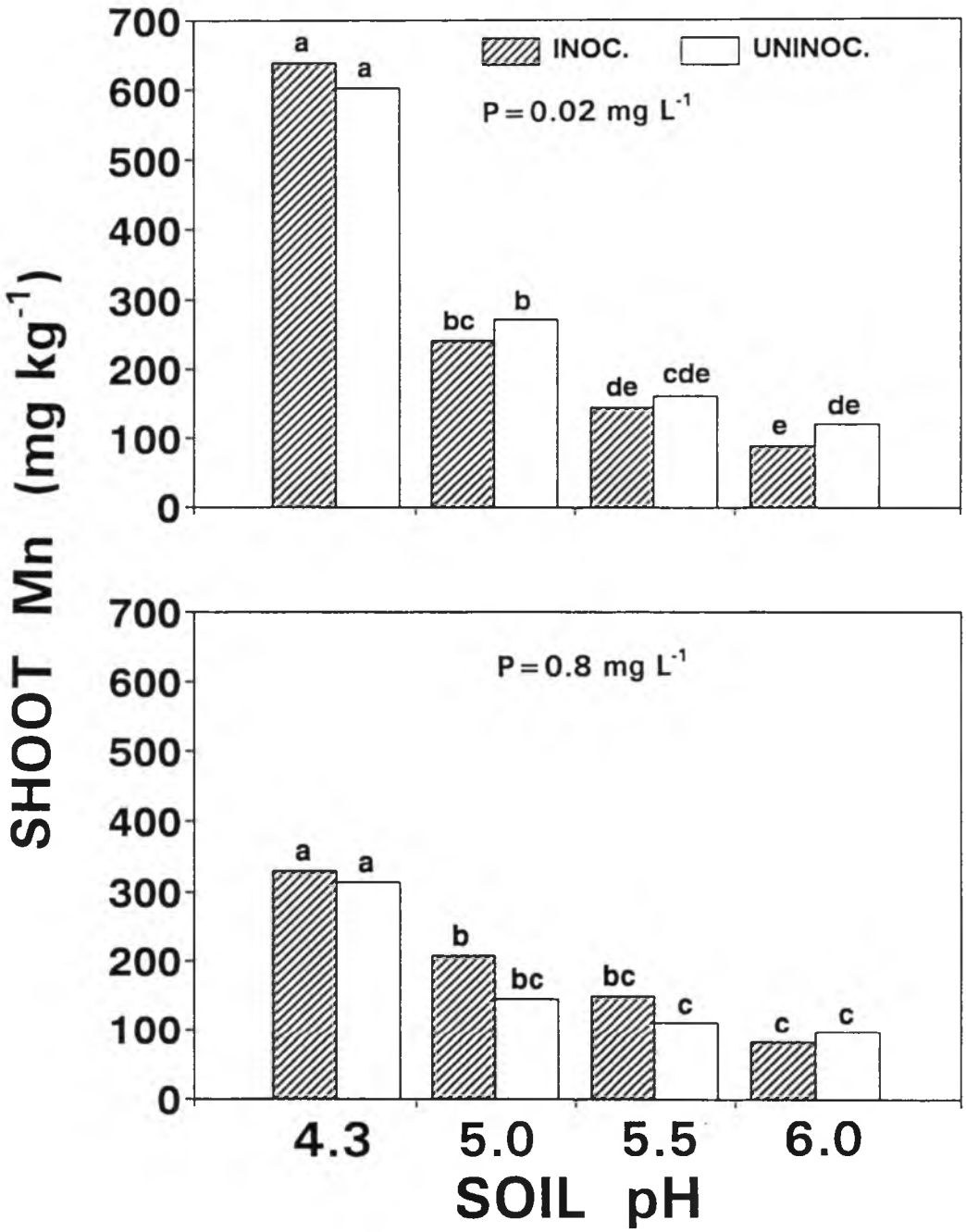

Fig.3.18. The influence of VAM inoculation, soil pH and P concentration on shoot Mn content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

With regards to shoot Ca of leucaena, mycorrhizal inoculation was beneficial when soil pH was higher than 4.3 and target soil P was 0.02 mg L^{-1} , but not at 0.8 mg P L^{-1} (Fig. 3.19). At the lower P concentration, shoot Ca content of leucaena grown at pH 6.0 was highest if soil was inoculated with *G. aggregatum*. Effect of soil pH and mycorrhizal inoculation on shoot Ca content of acacia (Fig. 3.20) was similar to their effect on shoot Ca content of leucaena. However, shoot Ca content did not increase as pH increased from 5.5 to 6.0. Inoculation with *G. aggregatum* caused higher shoot content of Ca in acacia than indigenous endophyte at pH's 5.5 and 6.0 with soil P concentration of 0.02 mg L^{-1}.

Magnesium (Mg) content of leucaena and acacia was influenced by soil pH and mycorrhizal inoculation at target P concentration of 0.02 mg L^{-1} but not at 0.8 mg L^{-1} (Figs. 3.21 and 3.22). At target P concentration of 0.02 mg L^{-1} shoot Mg content of leucaena increased if soil pH was higher than 4.3. Further increase was observed when soil was inoculated with *G. aggregatum*. However, at pH higher than 5.0 mycorrhizal inoculation did not increase shoot Mg content. Acacia grown in the inoculated soil with 0.02 mg P L^{-1} contained higher shoot Mg at pH 5.0 than at pH 4.3. Increasing pH to 5.5 did not significantly influence shoot Mg status. However, further increase in soil pH decreased shoot Mg content of acacia in the inoculated soil.

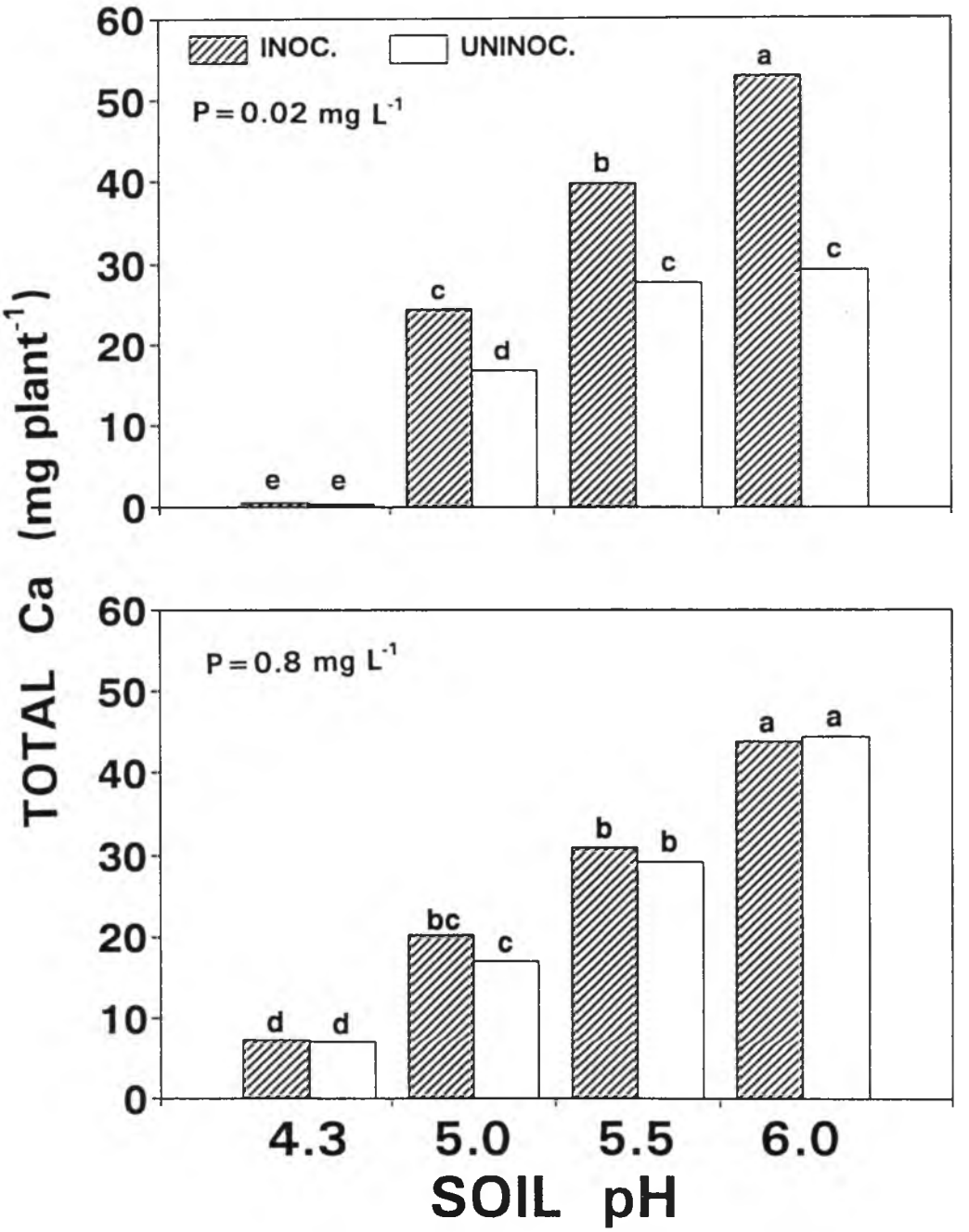

Fig.3.19. The influence of VAM inoculation, soil pH and P concentration on total shoot Ca content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

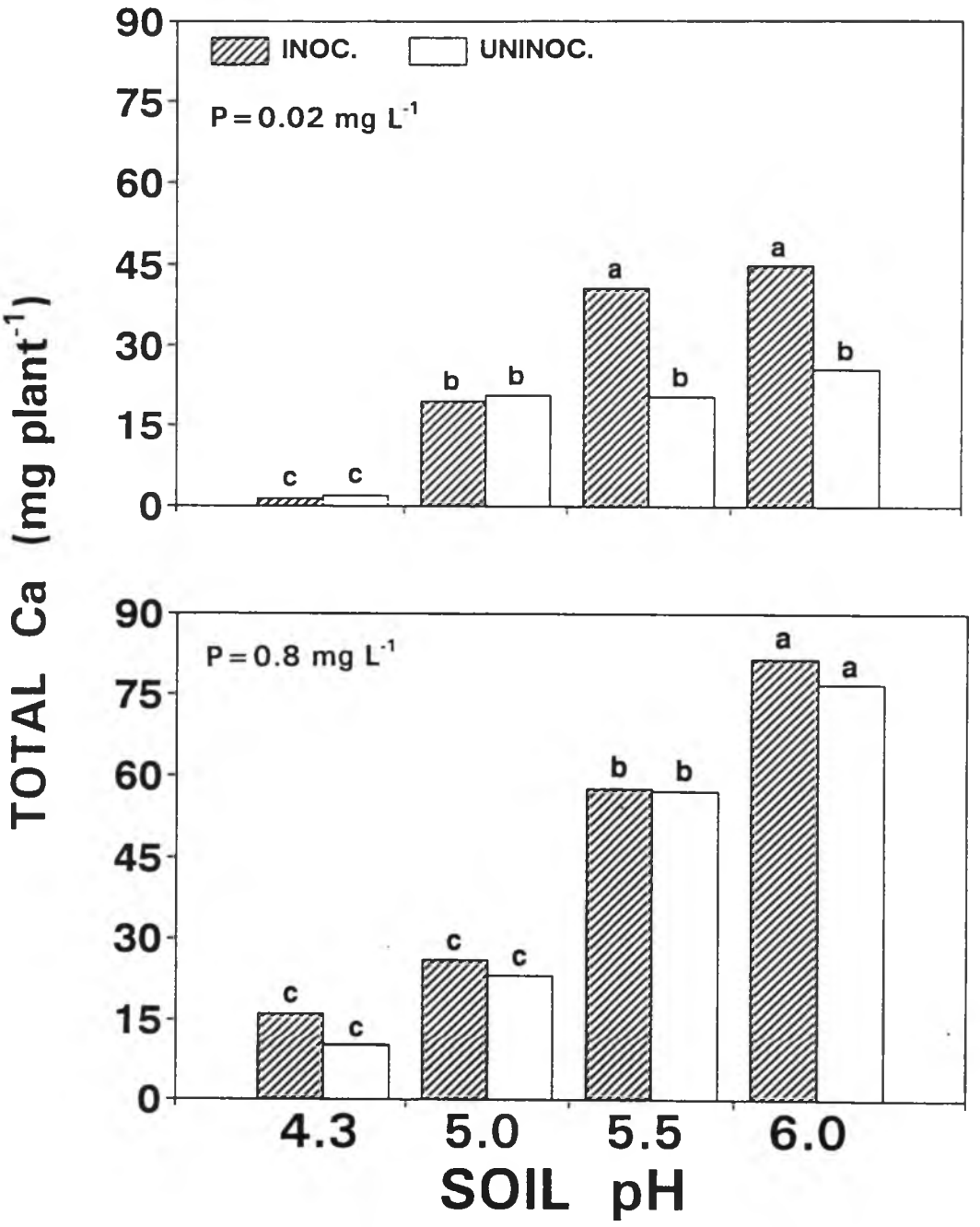

Fig.3.20. The influence of VAM inoculation, soil pH and P concentration on total shoot Ca content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

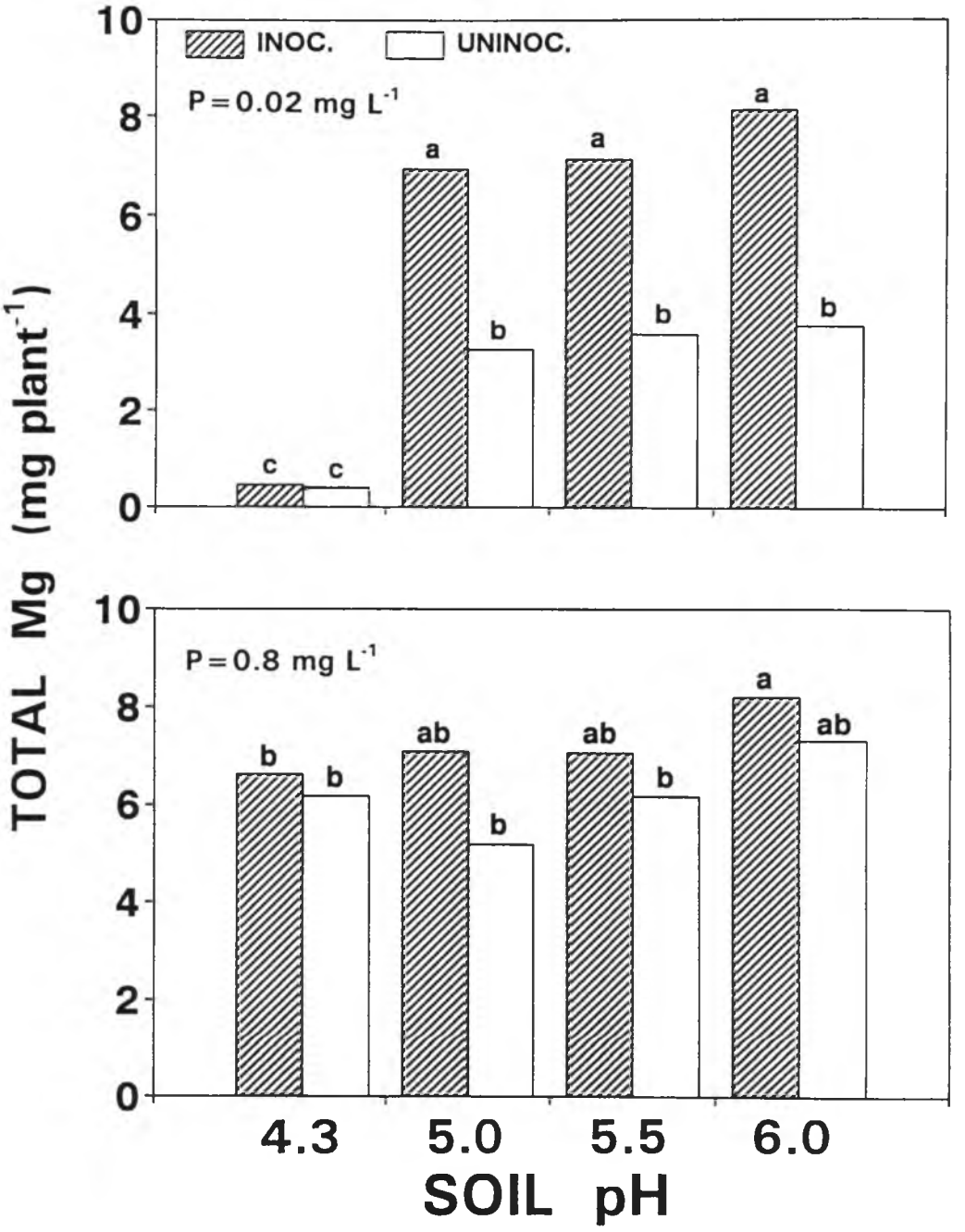

Fig.3.21. The influence of VAM inoculation, soil pH and P concentration on total shoot Mg content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

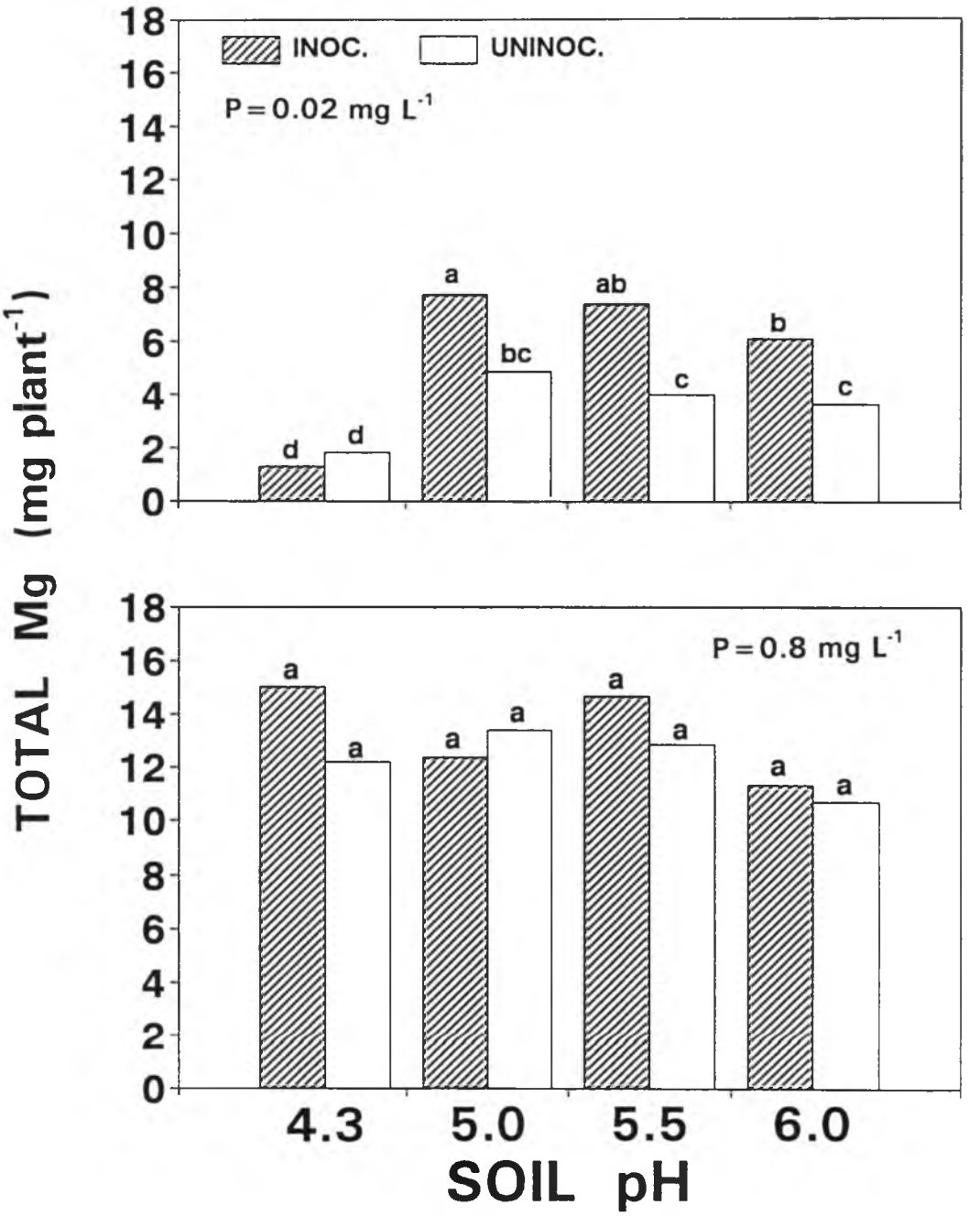

Fig.3.22. The influence of VAM inoculation, soil pH and P concentration on total shoot Mg content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

Shoot P of leucaena was significantly influenced by soil pH and mycorrhizal inoculation at both soil P concentrations tested, but shoot P status of acacia grown in soil with 0.8 mg P L^{-1} was not affected by VAM inoculation (Figs. 3.23, and 3.24). Highest shoot P content of leucaena was observed in the inoculated soil at pH 6.0 irrespective of soil P status. Similarly, The highest shoot P content of acacia grown in soil with 0.02 mg P L^{-1} was obtained when soil pH was raised 5.5. Further increase in pH did not significantly alter shoot P status in acacia.

DISCUSSION

Soil Chemical Properties Before Planting and After Harvest

Haynes and Swift (1985) noted that extractable Mn dropped drastically when soil pH was higher then 5.0. The soil used in the present experiment was an Oxisol with high total Mn content (Fox and Whitney, 1981). Manganese solubility decreases with increase in soil pH (Fox and Whitney, 1981; Fox *et al.*, 1985). At higher soil pH, Mn was precipitated as $Mn(OH)_2$ (Ritchie (1989). It can be concluded that raising soil pH to 5.0 is sufficient to mitigate the toxicity of Mn in the Wahiawa Oxisol for leucaena and acacia under these conditions.

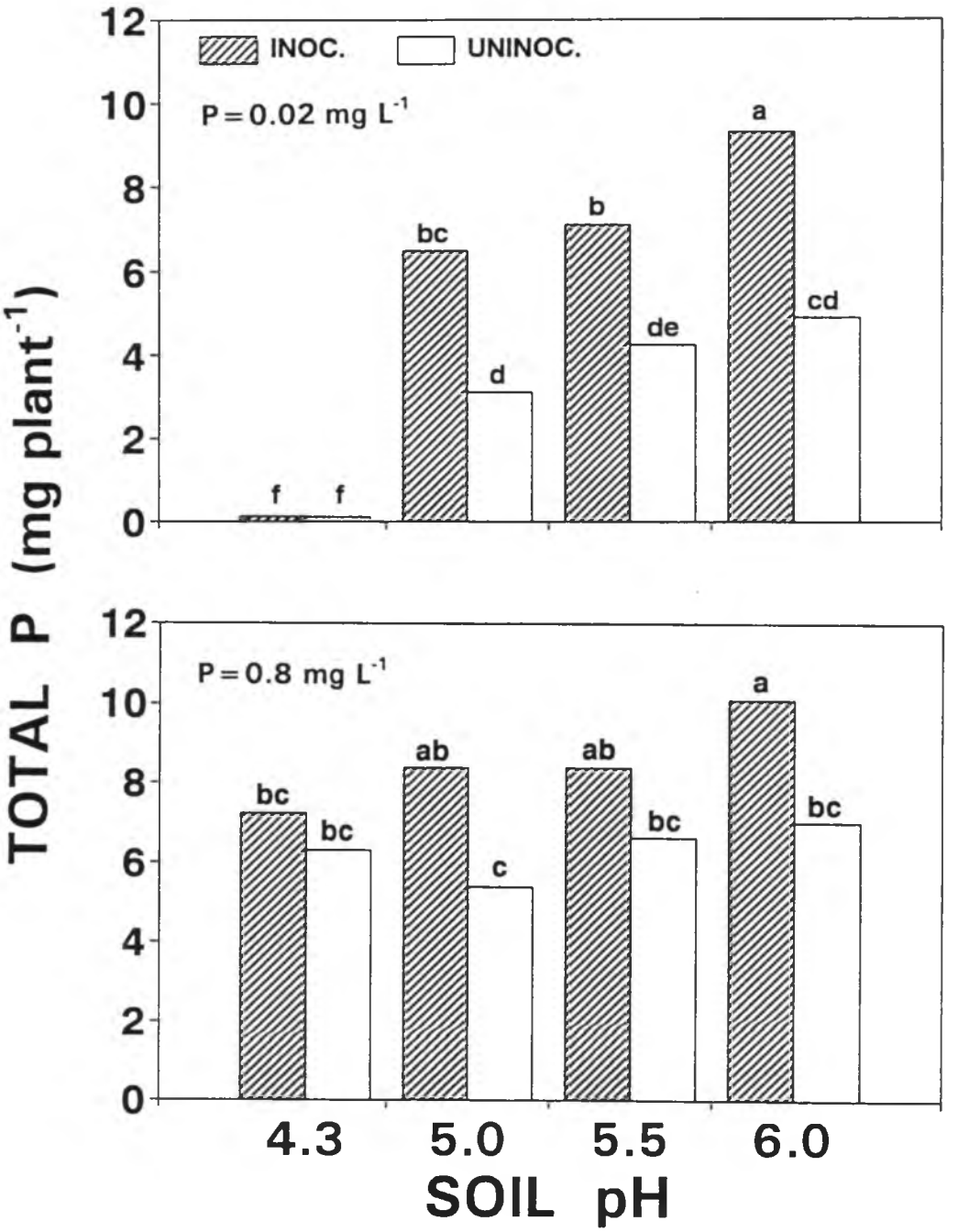

Fig.3.23. The influence of VAM inoculation, soil pH and P concentration on total shoot P content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

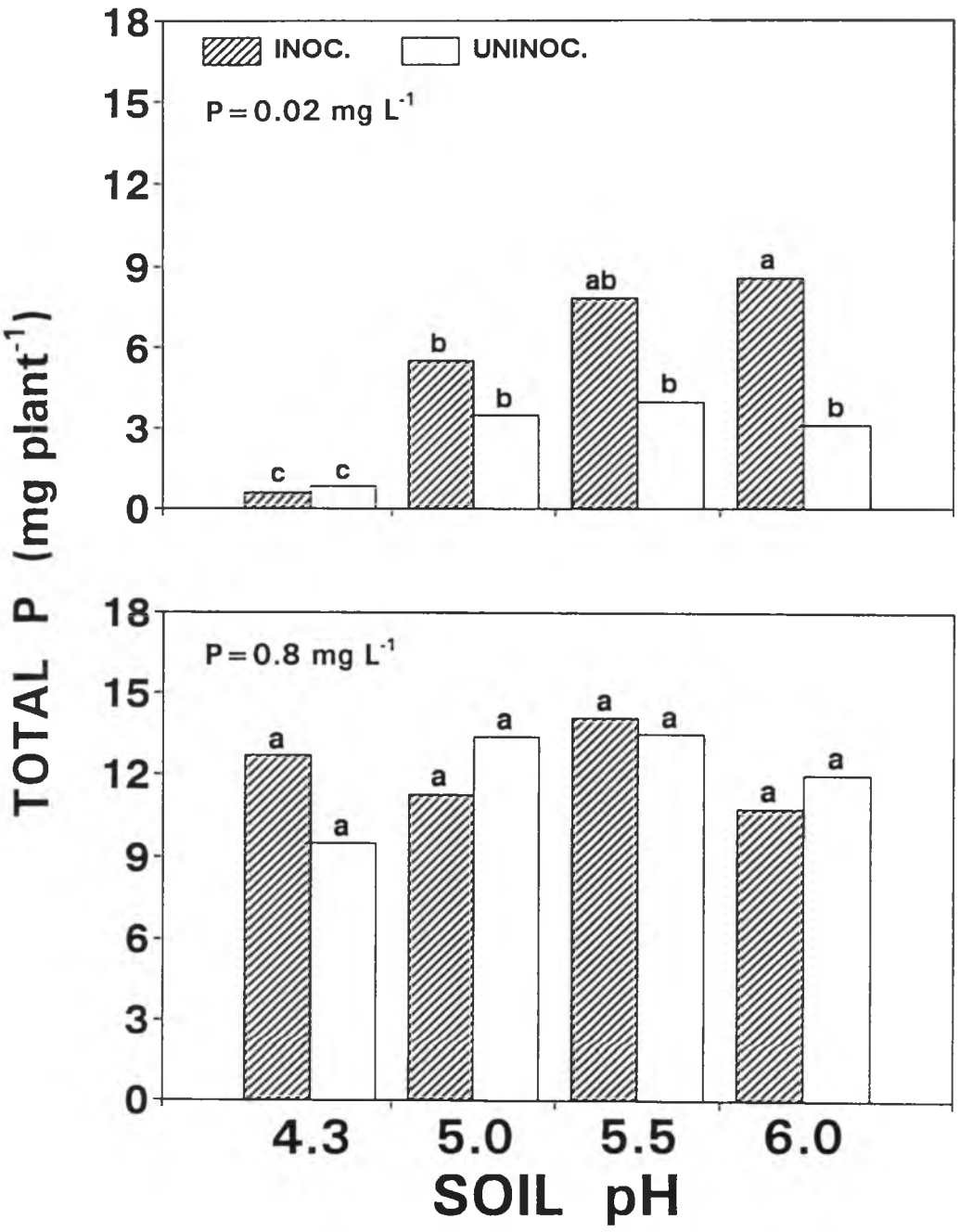

Fig.3.24. The influence of VAM inoculation, soil pH and P concentration on total shoot P content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

the lime source used in the present study was $Ca(OH)_2$ which readily dissociates into Ca^{+2} and $2OH^-$ in soil. As a result, soil pH as well as Ca content were increased by liming. An increase of soil pH and Ca after lime addition was also reported by other investigators (Naidu, 1986; Shamshuddin *et al.*, 1991; Syed-Omar *et al.*, 1991).

Soil used in the current study was an Oxisol which is variable charge soil. At pH 4.3, this soil is likely to be positively charged. Under this condition, an excess of anion $H_2PO_4^{-1}$ due to high P fertilizer will replace fixed OH^- at adsorption sites. The hydroxyl ion will go into the soil solution resulting in an increase in soil pH (Uehara and Gillman, 1981). The reduction in soil Mn at target pH of 4.3 in the soil with target P concentration of 0.8 mg L^{-1} is probably due to an increase in soil pH by this mechanism (see Table 3.2) and possibly due to the precipitation of Mn by excess phosphate anion thus reducing Mn availability (Norvell, 1988).

Vesicular-Arbuscular Mycorrhizal (VAM) Colonization

UNDERLINE

Vesicular-arbuscular mycorrhizal (VAM) colonization of roots of leucaena and acacia by the introduced or indigenous VAM endophytes was lower in the soil with the higher target soil solution phosphorus. The negative effect of high P on VAM colonization is well known (Yawney *et al.*, 1982; Hepper,

1983; Kucey and Diab, 1984; Siqueira *et al*. 1984; Wilson, 1984; Habte and Manjunath 1987; Koide and Mingguang Li, 1990). This phenomenon appears to be caused by an inhibition of mycorrhizal formation within roots (Braunberger *et al*. 1991). Mycorrhizal fungi require a certain amount of soluble carbohydrate (Kucey and Paul, 1982; Jakobsen and Rosendahl, 1990) and high P levels were found to reduce the concentration of soluble carbohydrate within roots (Same *et al*., 1983; Thompson *et al*., 1990).

Glomus aggregatum inoculum used in the present study readily infects roots of corn and produces abundant spores in crushed basalt (mansand) at pH 8.0 eventhough the fungus was isolated from soil with pH much below 7.0. Therefore, higher root colonization by *Glomus aggregatum* in soil of higher pH is likely to occur. However, the present study indicates that VAM colonization in acacia and leucaena by *G. aggregatum* in soil with 0.02 mg P L^{-1} was favored at pH 5.0-6.0. Thus, these results contradict the conclusions of Porter *et al*. (1987b) that VAM inoculation into soils in which they did not occur naturally will result in fewer infected roots and fewer spores than in their soil of origin. It is possible that *G. aggregatum* is adapted to a broad pH range. This phenomenon was supported by the finding of Hayman and Tavares (1985) who found that *G. mosseae* and *G. fasciculatum* colonized strawberry roots at pH 5 as well as at pH 7. Soil Mn at pH 5.0 to 6.0 was negligible (Table

3.1, and 3.2) and is unlikely to adversely affect mycorrhizal formation within the roots (Wang et al. 1985). Plants grew normally at pH 5.0 to 6.0; and sufficient carbohydrate must have been transported to roots for mycorrhizal colonization (Hayman, 1983). Therefore, soil pH may indirectly influence VAM colonization through its effect on plant growth. In the uninoculated soil, the low level of VAM colonization indicates that original soil contained low densities of infective propagules. Aziz and Habte (1989b) noted that lower quantities of infective propagules in an eroded Wahiawa soil compared to quantities of infective propagules in uneroded Wahiawa soil resulted in lower VAM colonization in the eroded soil.

The absence of mycorrhizal formation within leucaena and acacia roots may be related to high H^{+}, high Mn^{+2}, or low Ca^{+2} or combined effects of these cations. At pH 4.3, the concentration of H^{+} and Mn^{+2} were high and that of Ca^{+2} was low (Tables 3.1-3.2).

Development of Vesicular-Arbuscular Mycorrhizal (VAM) Activity

UNDERLINE

The present study revealed that the greater VAM activity associated with leucaena at pH 6.0 compared to pH 5.5 or 5.0 was not due to greater root colonization because VAM colonization levels at these pH's were similar (Figs.

3.1, and 3.2). The lack of relationship between mycorrhizal colonization and VAM effectiveness could be due to the fact that VAM colonization was observed after harvest (45 DAP). Manjunath and Habte (1988) found a positive correlation between VAM colonization and mycorrhizal effectiveness in leucaena during the first 35 DAP but not beyond this period.

Shoot Dry Weight

Shoot dry weight of leucaena was found to be in good agreement with the development of VAM activity and shoot P content at harvest but not with VAM colonization and root dry weight. Habte *et al.* (1987) found a positive correlation between VAM activity, monitored in terms of pinnule P content measured at regular intervals, and shoot P content of Leucaena. Other workers (Abbot and Robson 1985b; Habte and Aziz, 1991; Kucey and Diab, 1984) noted that increases in shoot P content were accompanied by increases in shoot dry weight. However, highest shoot dry weight was observed in leucaena grown in the inoculated soil at pH 6.0 even though mycorrhizal colonization at this pH was as high at pH's 5.0 and 5.5 (see figs. 3.1). The lack of agreement between VAM colonization and the growth of mycorrhizal plants was also observed in previous studies (Hayman and Tavares, 1985; Huang *et al.*, 1983). This lack of agreement between VAM colonization and plant growth is probably due to

difference in extramatrical hyphae by introduced VAM fungus at different soil pH. Higher growth of troyer citrange grown in soil inoculated with *Glomus* isolates from California than the growth of troyer citrange grown in soil inoculated with *Glomus* isolates from Florida was due to the ability of *Glomus* isolates from California to produce greater extramatrical hyphae (Graham *et al*. 1982).

Higher shoot dry weight of leucaena in the inoculated soil at pH 6.0 was not related to higher root dry weight. Since root dry weight at pH 6.0 was similar to that at pH 5.0 or 5.5, suggesting that greater extramatrical hyphae at this pH compared to pH's 5.0 and 5.5 caused higher uptake of nutrients (see Figs. 3.13-3.16, 3.19-3.24) and leucaena grew better accordingly.

At target soil P level of 0.02 mg L^{-1}, leucaena grew comparatively as at target soil P level of 0.8 mg L^{-1} if soil was inoculated with *G. aggregatum* (Fig. 3.7). However, acacia at soil P level of 0.02 mg L^{-1} failed to grow as normally as at higher soil P level even though the soil was inoculated with *G. aggregatum* (Fig. 3.8). At the lower soil P level, rhizosphere environment might be different when the soil was grown with different plant species, e.g., the production of exudates. Exudates contain carbon sources which are required for external hyphal growth (Paula and Siqueira, 1990). Soil grown with acacia at the lower P level may content less exudates than that grown with leucaena.

Consequently, external hyphal growth in soil grown with acacia might be less than that in soil grown with leucaena. External hyphae attached to the roots extend beyond the zone of phosphate depletion. Roots of plant colonized with mycorrhizal fungi that develop less hyphal will explore less available soil P than roots of plant colonized by mycorrhizal fungi that develop more external hyphae; as a result, growth rate was lower.

Acacia mangium is highly dependent on mycorrhizae (Habte, unpublished data). It has been reported that such host will not respond significantly to mycorrhizal inoculation if soil P is 0.2 mg L^{-1} (Habte and Manjunath, 1991). On the other hand, *Leucaena leucocephala* was categorized as very highly dependent on mycorrhizal fungi (Habte and Manjunath, 1991) and a significant response to mycorrhizal inoculation was observed eventhough soil P was established at a level sufficient for non-mycorrhizal plants (Habte and Manjunath, 1987; Habte and Manjunath, 1991). Thus, the difference of host plants to mycorrhizal dependence appears to be a reason for the lack of significant difference between shoot dry weight of acacia grown in the inoculated soil or in the uninoculated soil if soil P level was high but there was significant response to mycorrhizal inoculation in leucaena even though soil P concentration was high (0.8 mg P L^{-1}).

At pH 4.3, leaves of leucaena and acacia showed necrosis on their leaf margins as a result of Mn toxicity. Consequently, they did not grow normally (stunted). Leucaena used in the current study was reported to be sensitive to acid soil (Hutton 1981; Olvera, 1982; Balasundaran *et al.* 1988; and Halinda, 1988). On the other hand, acacia was reported to be tolerant to soil acidity (Glover and Heuveldop, 1986; and Halinda, 1988). The acid soil in which acacia was tolerant to acidity were high in Al (Glover and Heuveldop, 1985) and soil used in the present study was high in Mn. My data suggests that *Acacia mangium* may be sensitive to acid soil containing high concentrations of Mn.

The soil used in the current study was not sterilized; therefore mycorrhizal formation and mycorrhizal activity were observed (Figs. 3.1-3.6). However the effectiveness of indigenous endophytes was inferior to that of the introduced VAM fungus *G. aggregatum*. The inferiority of indigenous endophytes to *G. aggregatum* was shown by lower VAM colonization, lower development of mycorrhizal activity and lower mycorrhizal effectiveness measured in terms of shoot P content at harvest at pH's higher than 4.3. Such inferiority might also be due to inherently less effective indigenous endophytes. Aziz and Habte (1990); Habte and Aziz (1991) found inherently low effectiveness of indigenous endophytes in Wahiawa.

Toxic levels of Mn are most likely to control mycorrhizal development and effectiveness. At pH 4.3, high levels of Mn suppressed VAM formation within roots (Figs. 3.1, and 3.2) and thus the absence of mycorrhizal functioning at this pH (figs. 3.7 and 3.8). A deleterious effect of high Mn was previously demonstrated by Wang *et al.* (1985). However, Ca in the soil solution was low at pH 4.3. Besides toxic Mn; therefore, low Ca in the solution might have restricted VAM colonization and VAM functioning at pH 4.3.

Optimum growth of leucaena was found in inoculated soil with a pH 6.0 regardless of soil P levels while in acacia optimum growth was observed in the inoculated soil with pH's 5.0-6.0 and target P level of 0.02 mg L^{-1}, indicating that soil pH does not seem to directly influence effectiveness of mycorrhizal symbiosis, but plant species (Figs. 3.7 and 3.8). Acid sensitive plants such as *Leucaena leucocephala* var K8 grew normally at higher pH (pH 6.0 in the current study). More photosynthate (carbon source) will be produced by the better growing plants and more carbohydrates will be transported to the roots where mycorrhizae is formed. *Acacia mangium* will grow at low pH provided Mn toxicity does not occur. Thus sufficient carbon will be transfered to the roots even if soil pH is low. A consumption of carbon (photosynthate) by mycorrhizal fungi was documented by Kucey and Paul (1982); Jakobsen and Rosendhal (1990). Since VAM

colonization was similar at pH 5.0, 5.5, 6.0, it is hypothesized that carbon supply in leucaena was more adequate for the formation of extramatrical hyphae at pH 6.0. Extramatrical hyphae must have developed on the roots of acacia in soil at pH 5.0, 5.5, and 6.0 to the same extent. Higher growth enhancement of troyer citrange after VAM inoculation was related to more extramatrical hyphae formed (Graham *et al.*, 1982). Thus it can be concluded that the indirect effect of soil pH on the effectiveness of VAM symbiosis is related to the role of photosynthate plays in the development of extramatrical hyphae. However further research needs to be done to evaluate the rate of photosynthate production on formation of extramatrical hyphae.

Calcium content in the Wahiawa soil was inherently low and liming increased Ca in soil solution correspondingly (see table 3.1). Growth improvement due to mycorrhizal inoculation after lime addition might be due to increases in soil Ca content. The growth of leucaena and acacia was improved after soil was limed and inoculated with *G. aggregatum*. Soedarjo and Habte (in press) reported that greater growth of leucaena in inoculated Ultisol soil than in the uninoculated Ultisol soil after lime might be related to high Ca in the soil solution besides more appropriate pH.

Root Dry Weight

Salisbury and Ross (1985) reported that root growth is affected by plant P level. A study undertaken by Aziz and Habte (1987) also revealed that improved P uptake by associated plants after mycorrhizal inoculation enhanced root dry weight of cowpea. The present study shows an increase of shoot P in mycorrhizal leucaena (Fig. 3.23) but not in acacia grown in soil with 0.8 mg P L^{-1} (Fig. 3.24). Thus, higher root yield is due to higher P content of plant. The increase in shoot P of leucaena observed in the inoculated soil at pH 6.0 compared to that at pH 5.0 or 5.5 was not accompanied by higher root yield, indicating that a further increase in shoot P was not effective in increasing root dry weight. The lack of difference in root dry weight of acacia between inoculated and uninoculated soil at 0.8 mg P L^{-1} regardless of soil pH is due to the fact that internal P was similar (see Fig. 3.24).

Plant Height

Maximum plant height of leucaena was observed in the inoculated soil at pH 6.0 irrespective of initial soil P levels (see Fig. 3.11). This may be due to the fact that plants had the highest content of nutrients, especially P at that pH. Acacia grown in the inoculated soil with initial P

level of 0.02 mg L^{-1} was not taller at pH 5.5 than at pH 5.0, even though its P content was higher (Fig. 3.12). This may be due to the fact that acacia tends to grow laterally by increasing leaf size (visual observation).

Chemical Composition of Plants

It was reported that VAM fungi help plants take up more immobile micronutrients,i.e., Cu and Zn (Lambert et al., 1979; Manjunath and Habte, 1988; Habte and Aziz, 1991). The present study agrees with the results of these investigations. However, The present study showed that the effectiveness of mycorrhizal fungi in increasing these micronutrients is influenced by soil pH and host plants.

The similarity in shoot Mn content of leucaena and acacia irrespective of VAM colonization indicates that VAM fungi did not play a significant role in Mn uptake. These results disagree with the previous findings of Bethlenfalvay and Franson (1989) and Arines *et al*. (1989) who observed that mycorrhizal formation within roots of plants can reduce Mn uptake by associated plants.

Calcium and magnesium were mostly transported to the root zone by mass flow (Marschner, 1986) and consequently the higher Ca and Mg content in leucaena and acacia in the inoculated soil is not due to mycorrhizal formation. Lambert *et al*. (1979) noted that mycorrhizal colonization did not

directly increase the uptake of Ca and Mg but they may be required to balance negative charges resulting from higher P uptake by mycorrhizal plants. Therefore, higher Ca and Mg in leucaena grown in inoculated soil with pH 6.0 and in acacia grown in inoculated soil with pH of 5.5 may be due to higher P content in leucaena and acacia grown in inoculated soil than those grown in uninoculated soil (see Figs. 3.23 and 3.24).

The effect of soil pH on total shoot P paralleled to that of soil pH on the development of mycorrhizal activity which indicates a close relationship between VAM activity and total P uptake. Good agreement between the development of mycorrhizal activity and total P uptake was also found in a previous study (Habte et al., 1987).

Total P uptake of leucaena in inoculated soil at pH 6 was high regardless of soil P levels (Fig. 3.23). However, the effectiveness of introduced fungus in acacia was similar between target pH of 6.0 and 5.5 with initial P concentration of 0.02 mg L^{-1} (Fig. 3.24). Therefore, the results of the present study show that effectiveness of *G. aggregatum* is partly a function of indicator plants instead of the direct effect of soil pH. In addition, leucaena and acacia grew well at soil pH of 6.0 and 5.5 respectively. As a result, the plants were able to exploit more available and the presence of VAM fungus enhanced further P uptake.

CONCLUSIONS

In order to maximize VAM symbiosis in acid soils which are high in Mn, soil pH should be increased to reduce Mn toxicity. If an acid sensitive host plant is to be grown, further increase of soil pH beyond the pH needed to alleviate Mn toxicity may be required before the benefit of mycorrhizal inoculation is realized. No further increase of soil pH beyond the pH necessary to relieve Mn toxicity is required to obtain mycorrhizal-acid tolerant host plant symbiosis. Further studies need to be conducted to evaluate the role of Ca on mycorrhizal fungi.

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CHAPTER 4

EFFECT OF CALCIUM AMENDMENT ON VESICULAR ARBUSCULAR MYCORRHIZAE

INTRODUCTION

Infertility of acid soils may be related to low pH, deficiency of nutrients like P, Ca, Mo, and/or toxicity of Al, Mn or both (Marschner, 1991). A common means of improving fertility in these soils is lime addition. Lime can alleviate toxicity of Al and Mn, increase availability of some nutrients such as P, Ca and increase soil pH (Syed-Omar *et al.*, 1991; Haynes and Naidu, 1991). Liming acid soils was found to enhance the activity of beneficial soil microorganisms such as rhizobium (Balasundaran and Ali, 1988) and vesicular-arbuscular mycorrhizal (VAM) fungi (Aziz and Habte, 1989a). The stimulating effects of lime on beneficial soil microorganisms including VAM fungi could be due to more appropriate pH, increased availability of nutrients or alleviation of toxicity problems.

Several papers have been published on the beneficial effect of lime amendment on VAM symbiosis. For example, Hayman and Tavares (1985) found that enhancement of mycorrhizal effectiveness after lime addition was related to increase in pH. On the other hand, Siqueira *et al.* (1984) believed that improvement of VAM symbiosis due to liming was

related to Al detoxification. There has been very little effort to discuss in detail the effect of Ca on VAM effectiveness. An experiment done by Siqueira (1983) revealed a significant role of Ca on spore germination while another study by Elmes *et al.* (1980) revealed that Ca had a significant role in root colonization. Spore germination is the initial step of VAM colonization of roots. VAM colonization must first take place before fungi can increase nutrient uptake, because nutrient transfer from VAM fungus to roots of plants is mediated by arbuscules. In the first experiment, it was hypothesized that Ca might play role in improving mycorrhizal effectiveness. Therefore, the second experiment was conducted. The objective of this study was to determine if Ca or pH influences the effectiveness of mycorrhizal symbiosis in *L. leucocephala* or *A mangium* in the Wahiawa soil.

MATERIALS AND METHODS

Gypsum was used to establish calcium levels 0, 0.32, 0.96, and 1.61 g kg^{-1} soil. Calcium hydroxide which was included used for comparison was added at the rate of 0.0229 mole $Ca(OH)_2$ kg^{-1} soil which was equivalent to 1.61 g Ca kg^{-1} soil. Gypsum or lime was thoroughly mixed with soil and the mixture was incubated for 3 weeks before P was applied at approximately 60% of water holding capacity. Pots were

covered with brown paper and kept in the glasshouse of the Department of Agronomy and Soil Science at the University of Hawaii.

The soil used to grow leucaena had target P concentrations of 0.02 mg P L^{-1} and 0.8 mg P L^{-1}. A target P concentration of 0.02 mg L^{-1} was used to grow acacia because in the first experiment there was no significant difference in mycorrhizal effectiveness in the inoculated and uninoculated soils. The procedure of Fox and Kamprath (1970) was employed to establish these soil P concentrations. These target P levels were established three weeks after the soil had been amended with lime or gypsum and fifteen days before planting. Potassium phosphate monobasic was as the P source.

L. leucocephala (Lam) de Wit cv. K8 and *A. mangium* NFTA 276a were grown under natural light (21° 51'N and 156° 22'W) in the glasshouse of the Department of Agronomy and Soil Science at the University of Hawaii from February 10 to April 20, 1992. An average temperature during the experiment was 29.3°C.

The parameters measured to evaluate mycorrhizal response to soil pH were soil chemical properties before planting and after harvesting, plant height, VAM colonization and chemical composition of plants after harvest. Data collected were analyzed by using the SAS procedure (SAS Institute Inc., 1991).

RESULTS

Soil Chemical Properties Before Harvest

Table 4.1 depicts soil chemical properties before planting but after lime or gypsum addition. Liming caused a significant increase in soil pH, Ca and Mg content and reduced soil Mn significantly. The effect of gypsum was concentration dependent. At 0.32 g Ca kg^{-1} soil, soil pH was increased but Mn concentration in the soil solution was not altered. At higher Ca concentrations, pH decreased and Mn concentration increased. The concentration of Ca and Mg in the soil solution was increased significantly by gypsum amendment and the highest Ca and Mg concentrations were attained when the highest quantity of gypsum was added.

Soil Chemical Properties After Harvest

Table 4.2 depicts soil chemical properties after harvest of leucaena as influenced by an addition of lime or gypsum to soil with target P concentration of 0.02 mg L^{-1} and 0.8 mg L^{-1}. Soil pH did not change compared to that before harvest but soil Mn, Ca, and Mg increased in general. Mn did not increase if soil was untreated, limed or amended with the lowest amount of gypsum; soil Ca level was highest in soil amended with the highest amount of gypsum.

Table 4.1. Effect of lime or gypsum addition on soil chemical properties before planting.

Treatment (mg Ca Kg^{-1} soil)	pH (2:1) H_2O	pH (2:1) 0.01 M $CaCl_2$	Nutrients in soil solution Mn	Ca	Mg
			------ mg L^{-1}--------		
Untreated	4.91 c	4.26	1.84 c	6.05 d	2.60 d
Gypsum $\{Ca(SO)_4\}$:					
0.32	5.06 b	4.79	2.40 c	19.80 d	14.30 c
0.96	4.76 d	4.72	7.55 b	179.80 b	19.60 b
1.61	4.66 e	4.69	10.26 a	629.00 a	26.60 a
L = lime $\{Ca(OH)_2\}$:					
1.61	5.99 a	5.37	0.97 d	67.30 c	13.15 c

Figures in the same column with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

Soil pH (after harvest of leucaena) in soil with higher P concentration was higher than in soil with a target P level of 0.02 mg L^{-1} (Table 4.2). These pH increases were accompanied by decreases in Mn concentration, the greatest decrease occurred in the lime soil. At this P level, amendment of lime and gypsum increased Ca and Mg in the soil solution.

Table 4.2. Effect of lime, gypsum or P concentration on soil chemical properties after harvest of leucaena.

Treatment (mg Ca kg^{-1} soil)	Soil pH (1:2 H_2O)	Nutrients in soil solution (mg L^{-1}) Mn	Ca	Mg
	---------- at 0.02 mg P L^{-1} ----------			
Untreated	5.0 c	6.8 c	25.9 d	64.7 c
Gypsum $\{Ca(SO)_4\}$:				
0.32	5.1 b	3.1 c	39.5 d	28.5 d
0.96	4.8 d	14.5 b	263.0 b	88.9 b
1.61	4.7 e	20.9 a	582.5 a	102.4 b
L = lime $\{Ca(OH)_2\}$:				
Lime (L)	5.8 a	ND	154.9 c	59.7 c
	---------- at 0.8 mg P L^{-1} ----------			
0	5.7 b	0.2 c	5.8 e	13.7 c
Gypsum $\{Ca(SO)_4\}$:				
0.32	5.6 c	0.4 c	24.6 d	27.8 b
0.96	5.3 d	3.1 b	44.9 c	32.9 b
1.61	5.2 e	5.6 a	187.8 a	62.9 a
L = lime $\{Ca(OH)_2\}$:				
Lime (L)	6.1 a	ND	67.3 b	28.6 b

ND= not detected. Figures in the same column under different P concentration with the same letter are not significantly different at the 5% probability level by the L.S.D. test.

The changes in soil chemical properties observed following acacia growth followed the same trend to as those observed after leucaena was harvested (Table 4.3). Soil pH

was increased by addition of lime or by the lowest amount of gypsum, while higher rates of gypsum caused pH to decrease. Mn concentration in untreated soil was high and a typical Mn toxicity symptom were observed, necrosis on the leaf edges of acacia. When lime was added, Mn concentration was reduced to a non-toxic level. Addition of the lowest amount of gypsum reduced Mn solubility in the untreated soil by about 50% which was not toxic to acacia. When higher concentrations of gypsum were added, Mn solubility as well as the solubility of Ca and Mg were enhanced.

Table 4.3. Effect of lime, gypsum or P concentration on soil chemical properties after harvest of acacia.

Treatment (mg kg^{-1} soil)	Soil pH (1:2 H_2O)	Nutrients in soil solution (mg L^{-1}) Mn	Ca	Mg
Untreated	4.68 c	15.4 b	41.2 d	53.8 b
Gypsum $\{Ca(SO)_4\}$:				
0.32	4.80 b	8.2 c	70.8 d	65.9 b
0.96	4.61 d	38.7 a	477.2 b	121.4 a
1.61	4.54 d	43.3 a	649.8 a	115.6 a
L = lime $\{Ca(OH)_2\}$:				
Lime (L)	5.30 a	3.2 c	265.0 c	69.9 b

ND= not detected. Figures in the same column with the same letter are not significantly different at 5% probability level by the L.S.D. test.

Vesicular-Arbuscular Mycorrhizal (VAM) Colonization

The highest level of VAM colonization in leucaena roots was observed in the inoculated soil with 0.02 mg P L^{-1} amended with lime or the lowest amount of gypsum (Fig. 4.1). The influence of these treatments on VAM colonization was similar. Higher quantities of gypsum deleteriously affected VAM colonization. In the soil with 0.8 mg P L^{-1}, VAM colonization in leucaena roots in inoculated soil was not different from that in uninoculated soil. At this P level, the higher concentrations of gypsum also were deleterious to VAM colonization. No mycorrhizal colonization was observed in the untreated soil.

Similarly, lime or gypsum stimulated VAM colonization in acacia roots (Fig. 4.2). However, the highest VAM colonization was observed at limed soil and inoculated with *G. aggregatum*. Gypsum applied at quantities exceeding 0.32 g Ca kg^{-1} was detrimental to VAM colonization and resulted in significant difference between inoculated and uninoculated soil. Acacia roots were not colonized by VAM fungi in the untreated soil.

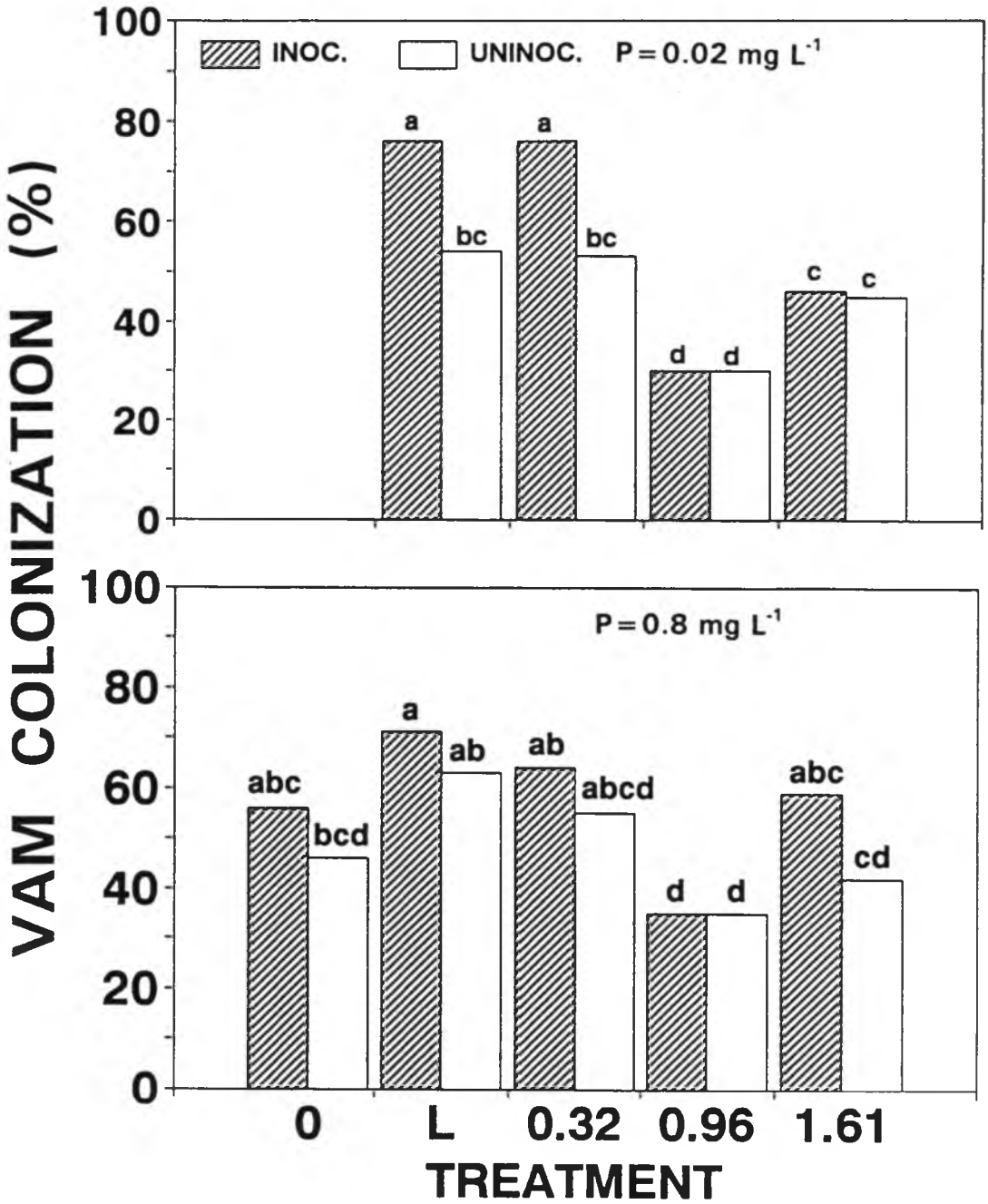

Fig. 4.1. The influence of VAM inoculation, lime or gypsum and P concentration on VAM colonization of leucaena roots. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

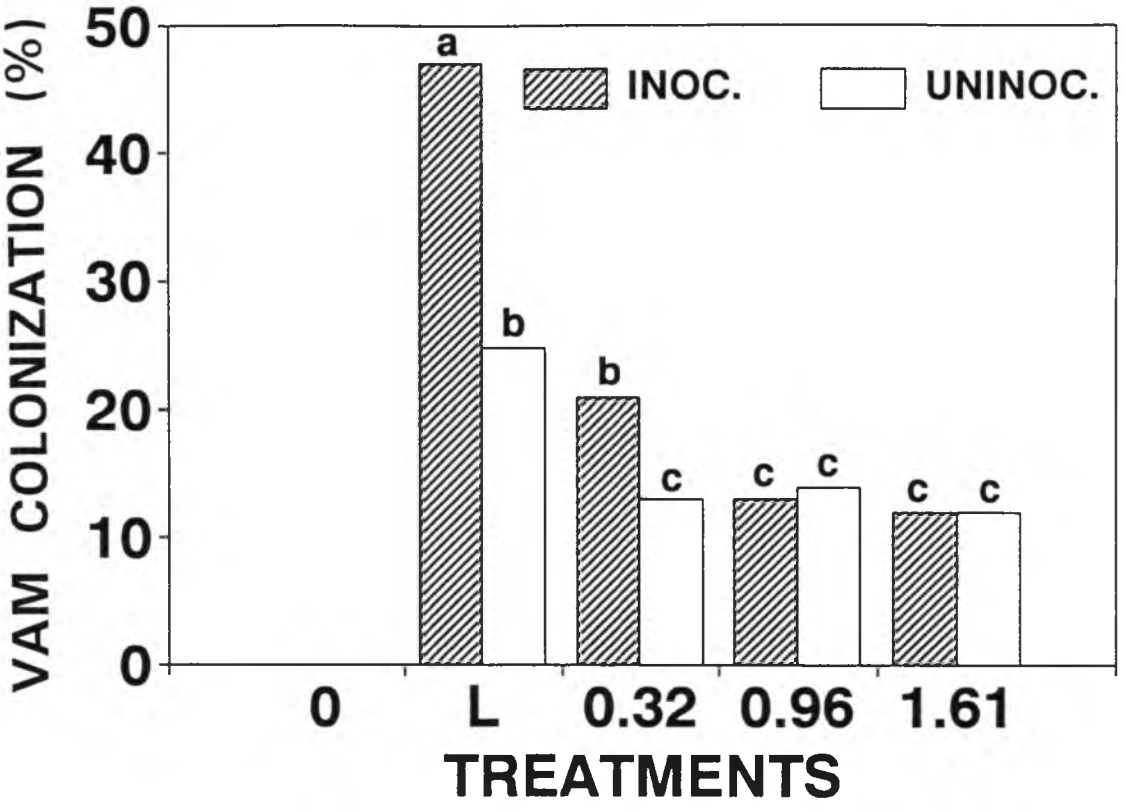

Fig. 4.2. The influence of VAM inoculation and lime or gypsum on VAM colonization of acacia roots. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

Development of Vesicular-Arbuscular Mycorrhizal (VAM) Activity

In soil with 0.02 mg P L^{-1}, no mycorrhizal activity was detected if the soil was not amended with lime or the lowest concentration of gypsum. At higher gypsum concentrations, VAM activity was comparable in inoculated and uninoculated soil. Mycorrhizal activity in the limed soil or in the soil amended with the lowest quantity of gypsum peaked at 50 DAP. At the higher soil P concentration, pinnule P status of plants in inoculated soil was significantly different from that in uninoculated soil only if in the presence of lime (Fig. 4.4). Mycorrhizal activity in this soil peaked at the same time as that in limed or gypsum amended soil (0.32 g Ca kg^{-1}) with 0.02 mg P L^{-1}.

No mycorrhizal activity was observed in acacia grown in the untreated soil (Fig. 4.5). Here also, mycorrhizal activity in inoculated and uninoculated soil, did not differ significantly if gypsum was applied at a rate higher than 0.32 g Ca kg^{-1}. Only amendment of lime or the lowest gypsum caused higher VAM activity in soil inoculated with *G. aggregatum* than in uninoculated soil. The peak of mycorrhizal activity was not noticed until 45 DAP.

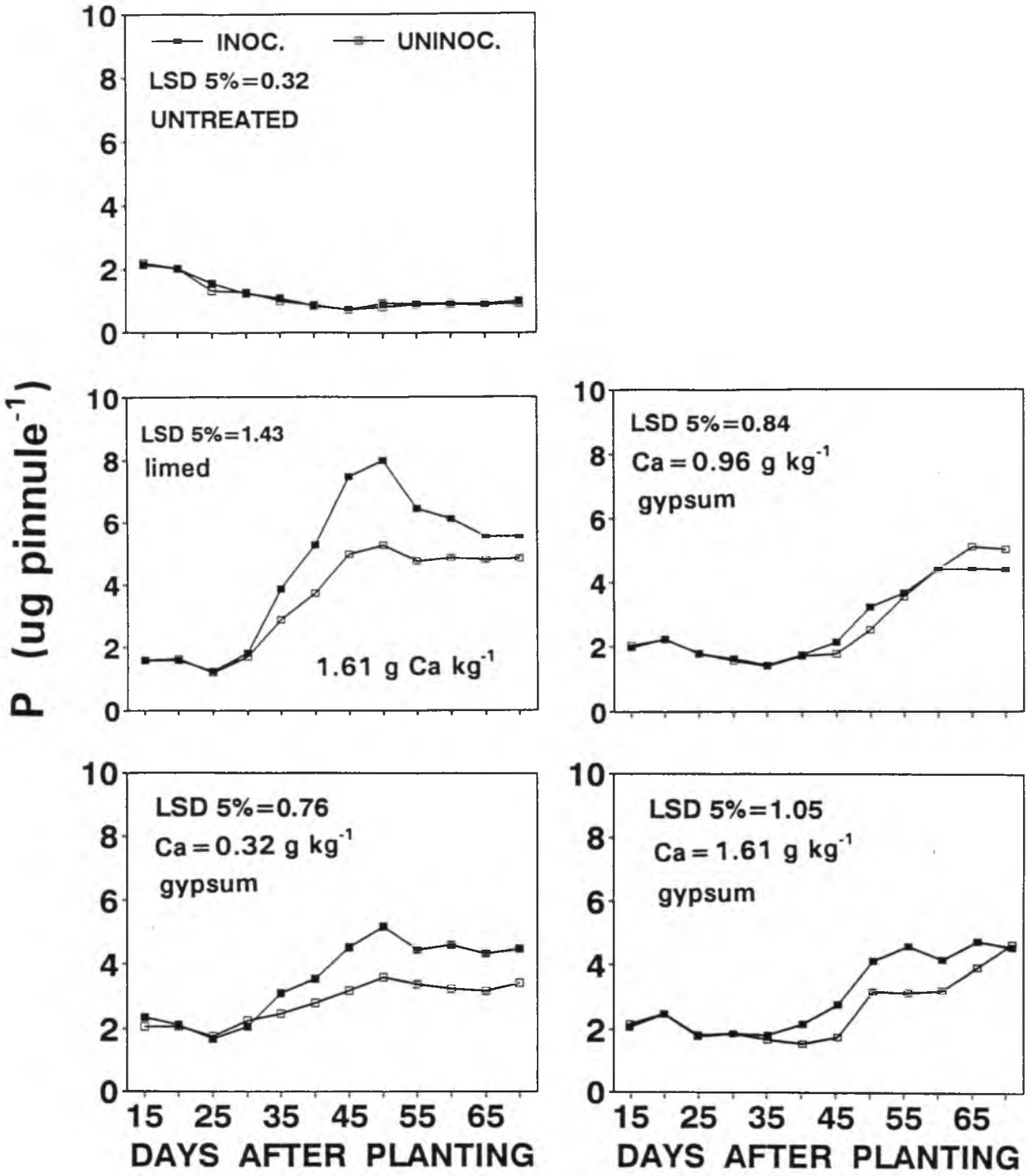

Fig. 4.3. The influence of VAM inoculation and lime or gypsum on the development of VAM activity in leucaena grown in soil with 0.02 mg P L^{-1}.

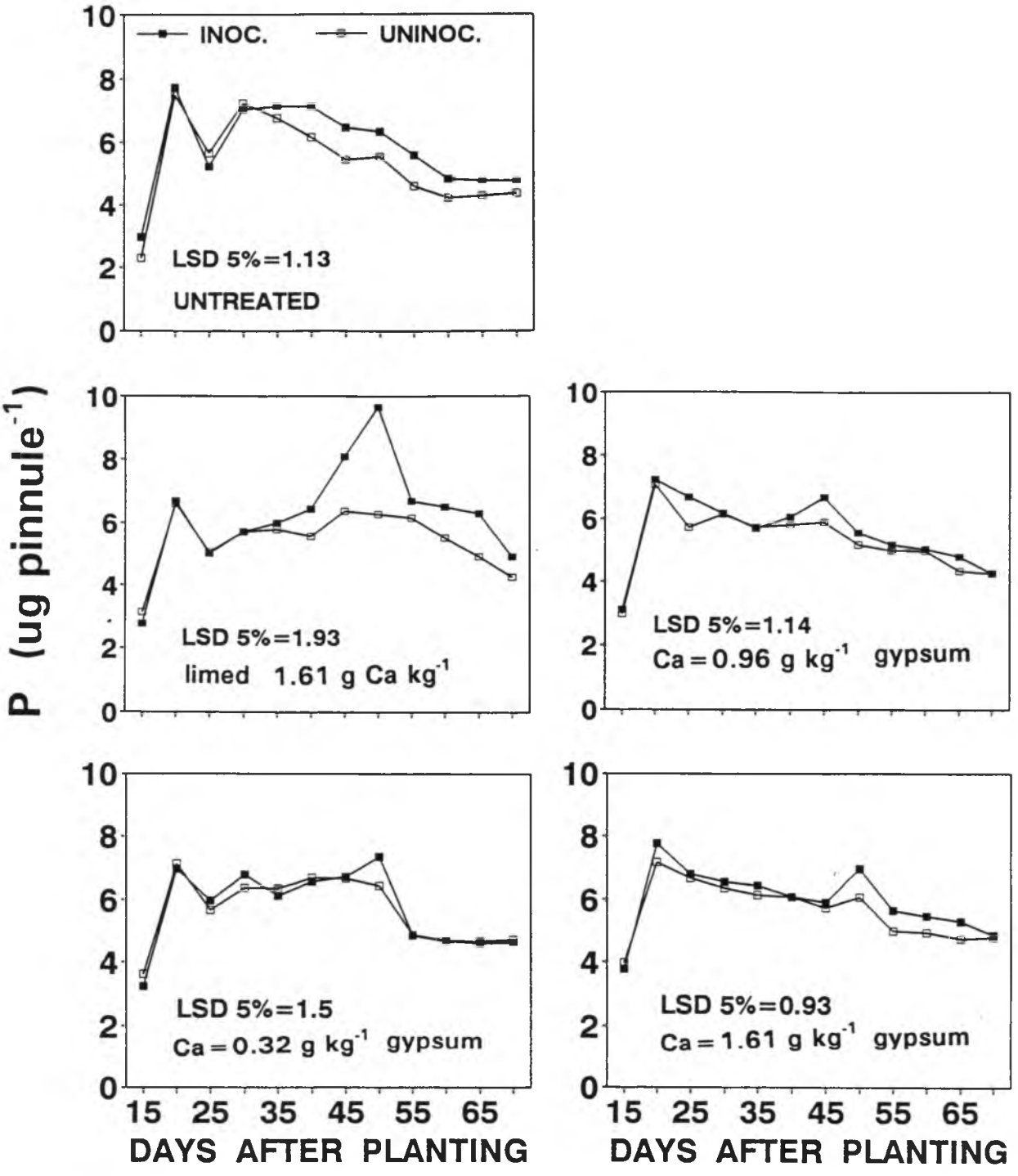

Fig. 4.4. The influence of VAM inoculation and lime or gypsum on the development of VAM activity in leucaena grown in soil with 0.8 mg P L^{-1}.

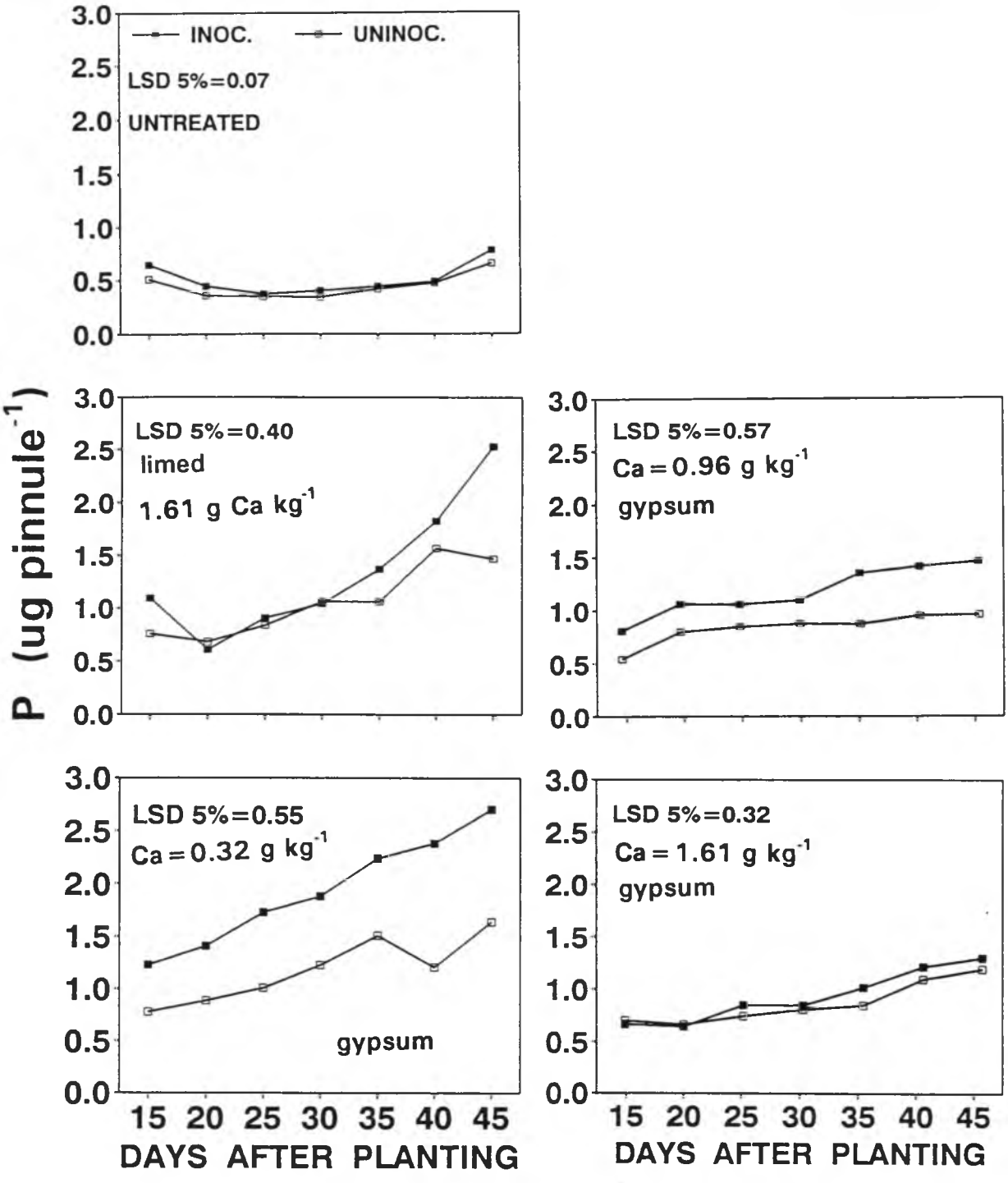

Fig. 4.5. The influence of VAM inoculation and lime or gypsum on the development of VAM activity in acacia grown in soil with 0.02 mg P L^{-1}.

Shoot Dry Weight

Influence of lime or gypsum addition on shoot dry weight of leucaena grown in soil with 0.02 mg P L^{-1} or with 0.8 mg P L^{-1} is shown in Figure 4.6. At the lower level of P, inoculation with *G. aggregatum* stimulated shoot dry weight of leucaena when the soil was limed or amended with gypsum. However, the highest shoot dry weight of leucaena was observed in inoculated soil amended with lime. At the higher level of P, mycorrhizal inoculation increased shoot dry weight of leucaena only in limed soil. Liming and mycorrhizal inoculation increased shoot dry weight by 47% and 24% at low P and high P, respectively. Thus high P diminished the mycorrhizal inoculation effect.

Figure 4.7 depicts the influence of lime or gypsum addition and mycorrhizal inoculation on shoot dry weight of acacia. Amendment with lime or gypsum enhanced shoot dry weight of acacia, and shoot dry weight was further enhanced when the soil was inoculated with *G. aggregatum*, suggesting less effective indigenous endophytes compared to the introduced one. The highest shoot dry weight was observed in the inoculated soil amended with the lowest amount of gypsum.

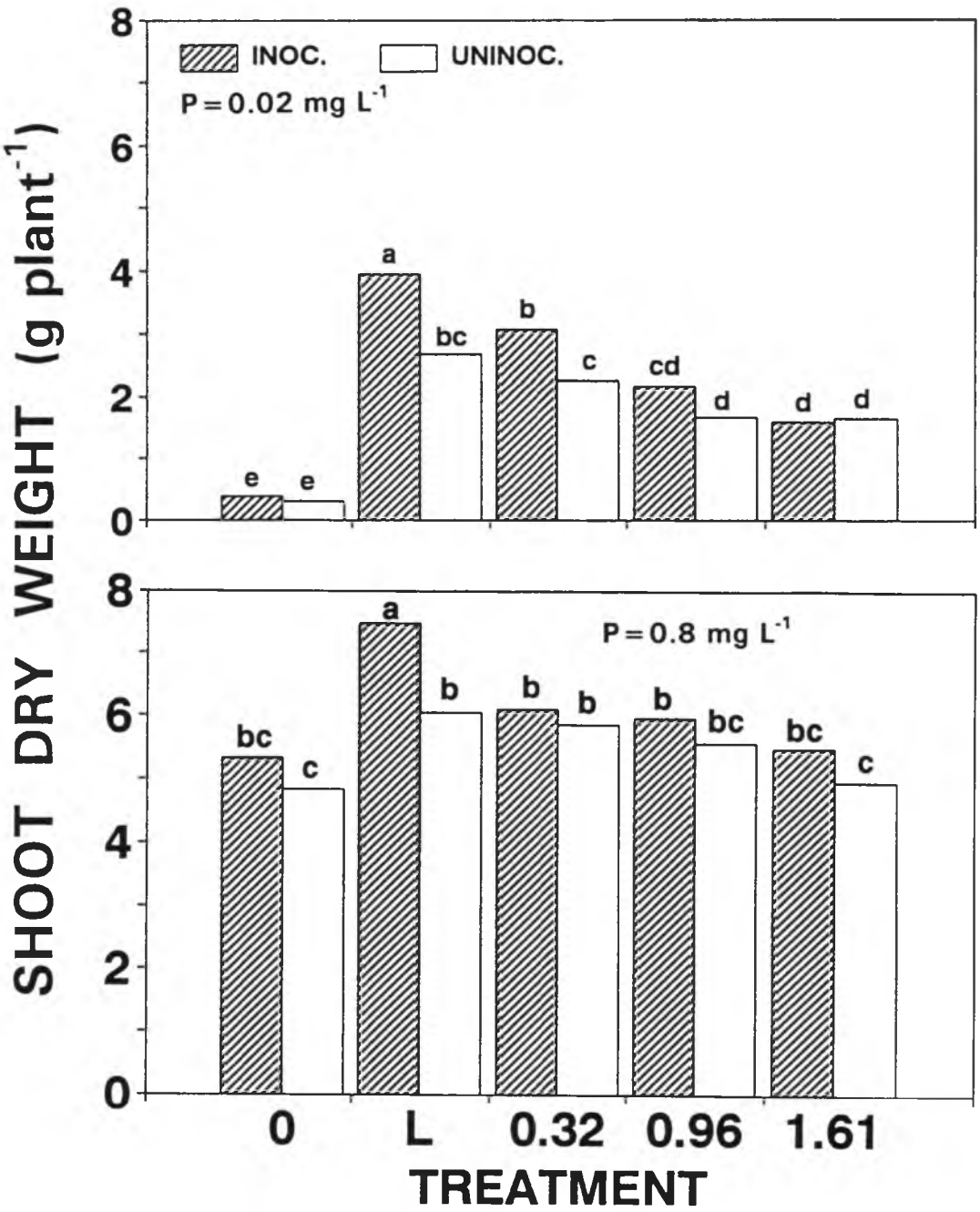

Fig. 4.6. The influence of VAM inoculation, lime or gypsum and P concentration on shoot dry weight of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

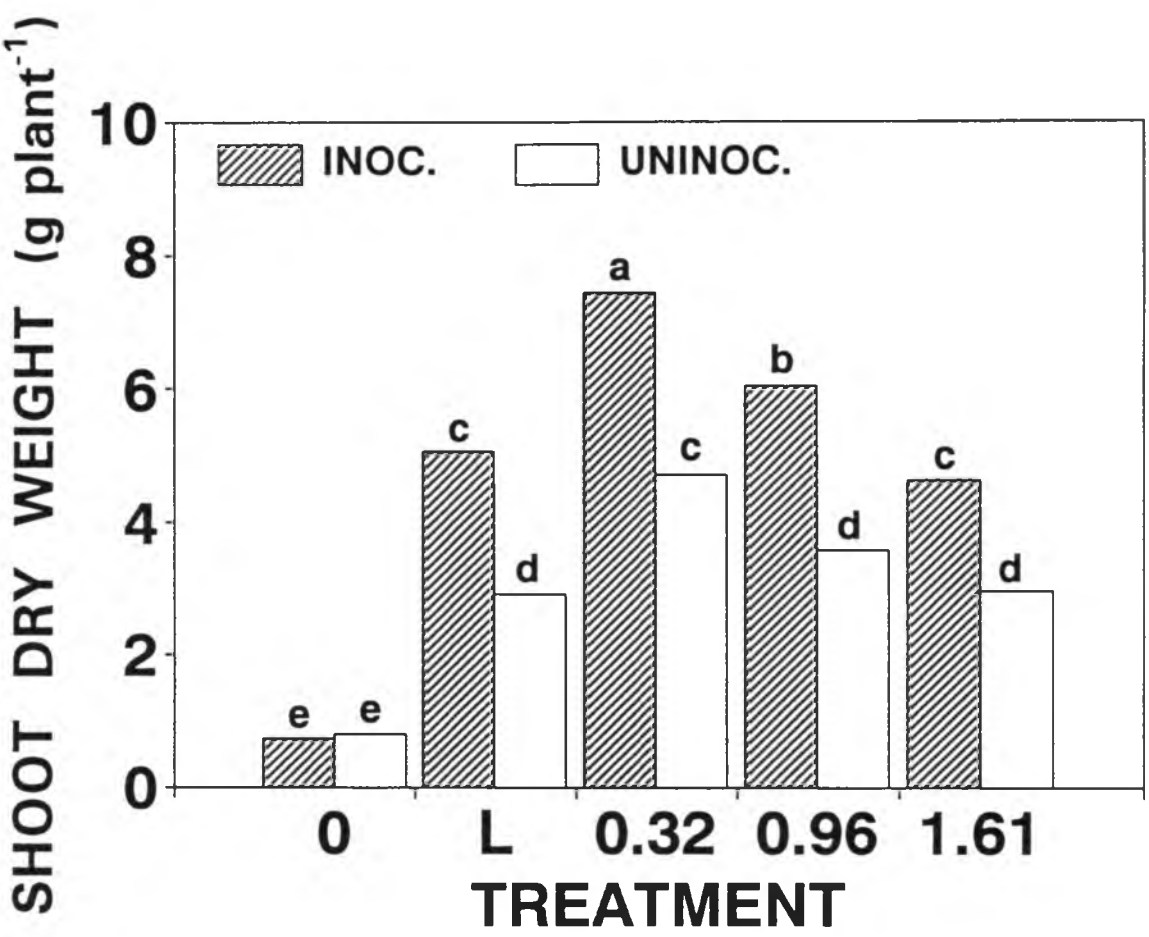

Fig. 4.7. The influence of VAM inoculation and lime or gypsum on shoot dry weight of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

Root Dry Weight

In soil with the target P level of 0.02 mg L^{-1}, the highest root dry weight was obtained by amending soil with lime and inoculating it with *G. aggregatum*, but it was not significantly different from that of leucaena in inoculated soil amended with the lowest level of gypsum (Fig. 4.8). At concentrations of gypsum higher than 0.32 g Ca kg^{-1}, mycorrhizal inoculation did not result in higher root dry weight than indigenous endophytes. At higher soil P level, only in limed soil did mycorrhizal inoculation stimulate root dry weight.

Mycorrhizal inoculation in soil amended with lime or the first two increments of gypsum enhanced root dry weight of acacia significantly (Fig. 4.9). The highest root dry weight of acacia was obtained when the soil was amended with the lowest level of gypsum and inoculated with *G. aggregatum*.

Plant Height

At 15 DAP, plant heights of leucaena or acacia were not affected by treatments (Fig. 4.10 and 4.11) which means that leucaena and acacia did not respond to lime or gypsum addition at the early stage. Until this time, the plants appeared to be supported by food reserve in the seed. At 65

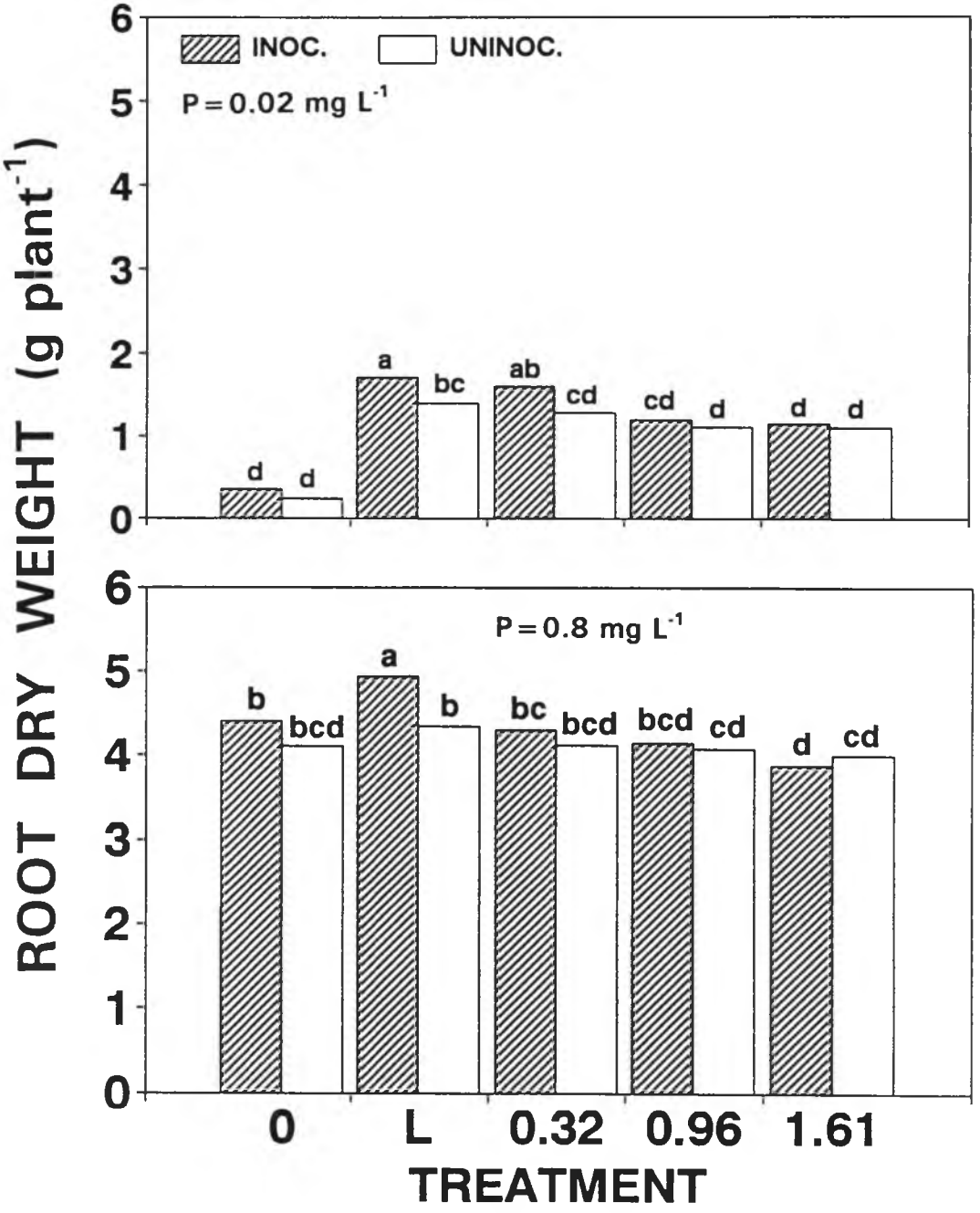

Fig. 4.8. The influence of VAM inoculation, lime or gypsum and P concentration on root dry weight of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

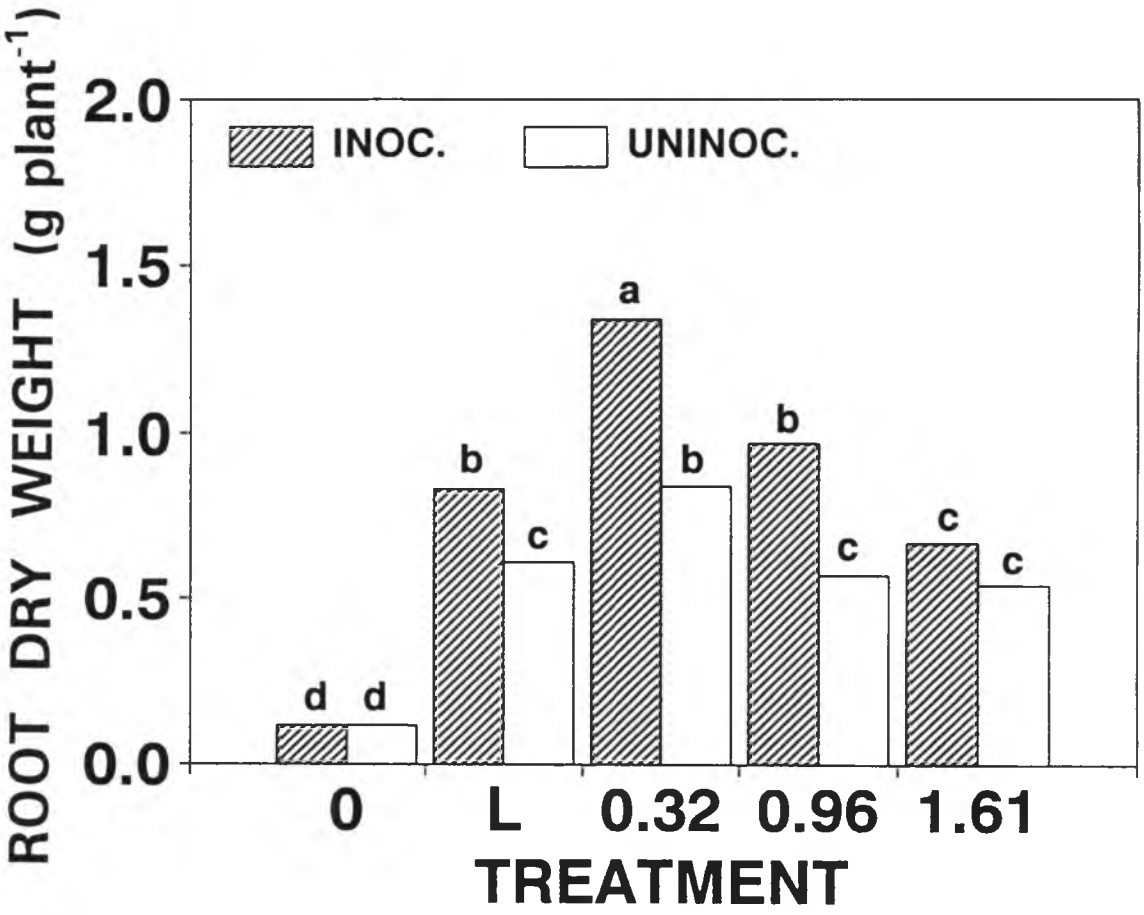

Fig. 4.9. The influence of VAM inoculation and lime or gypsum on root dry weight of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

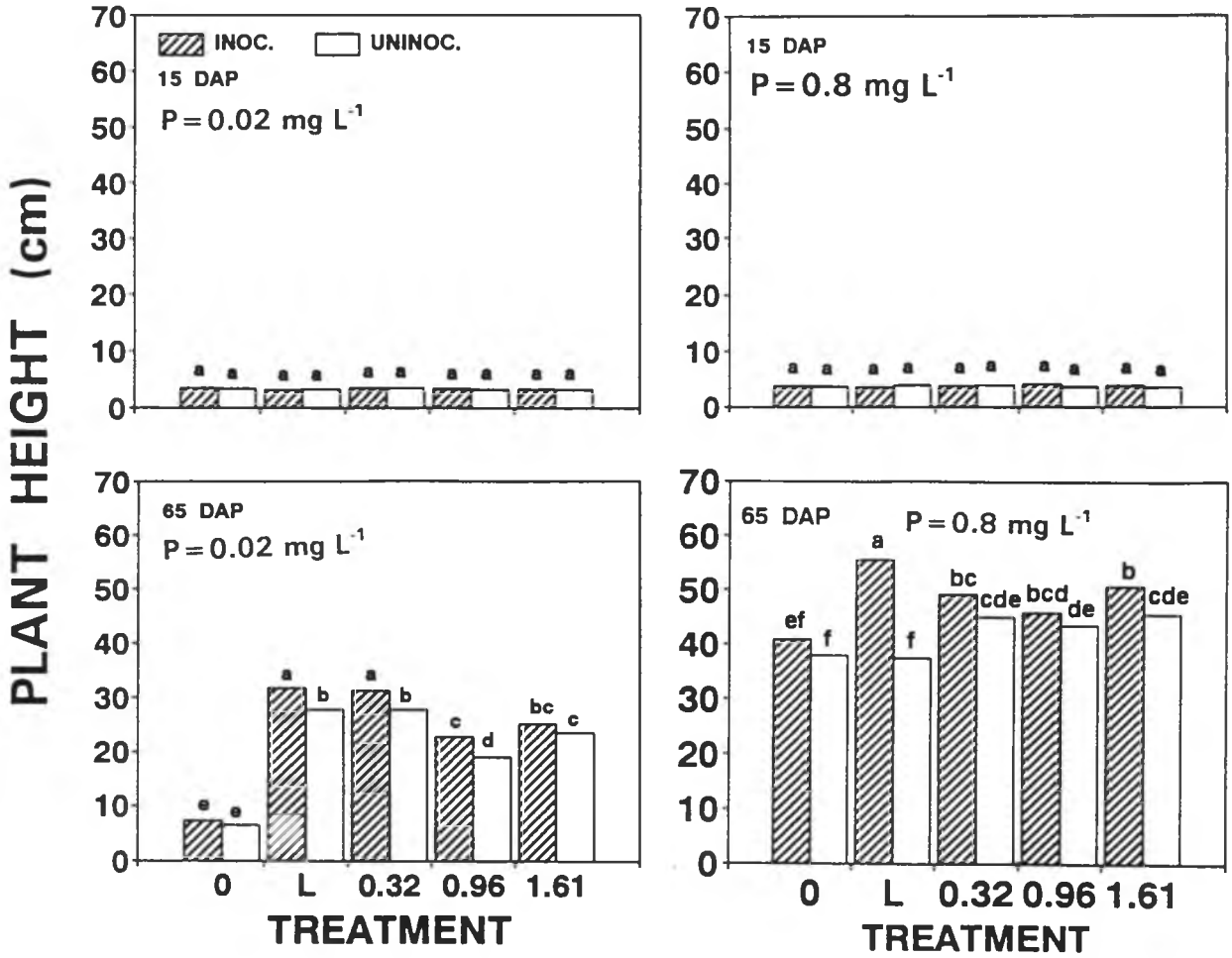

Fig. 4.10. The influence of VAM inoculation, lime or gypsum and P concentration on plant height of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

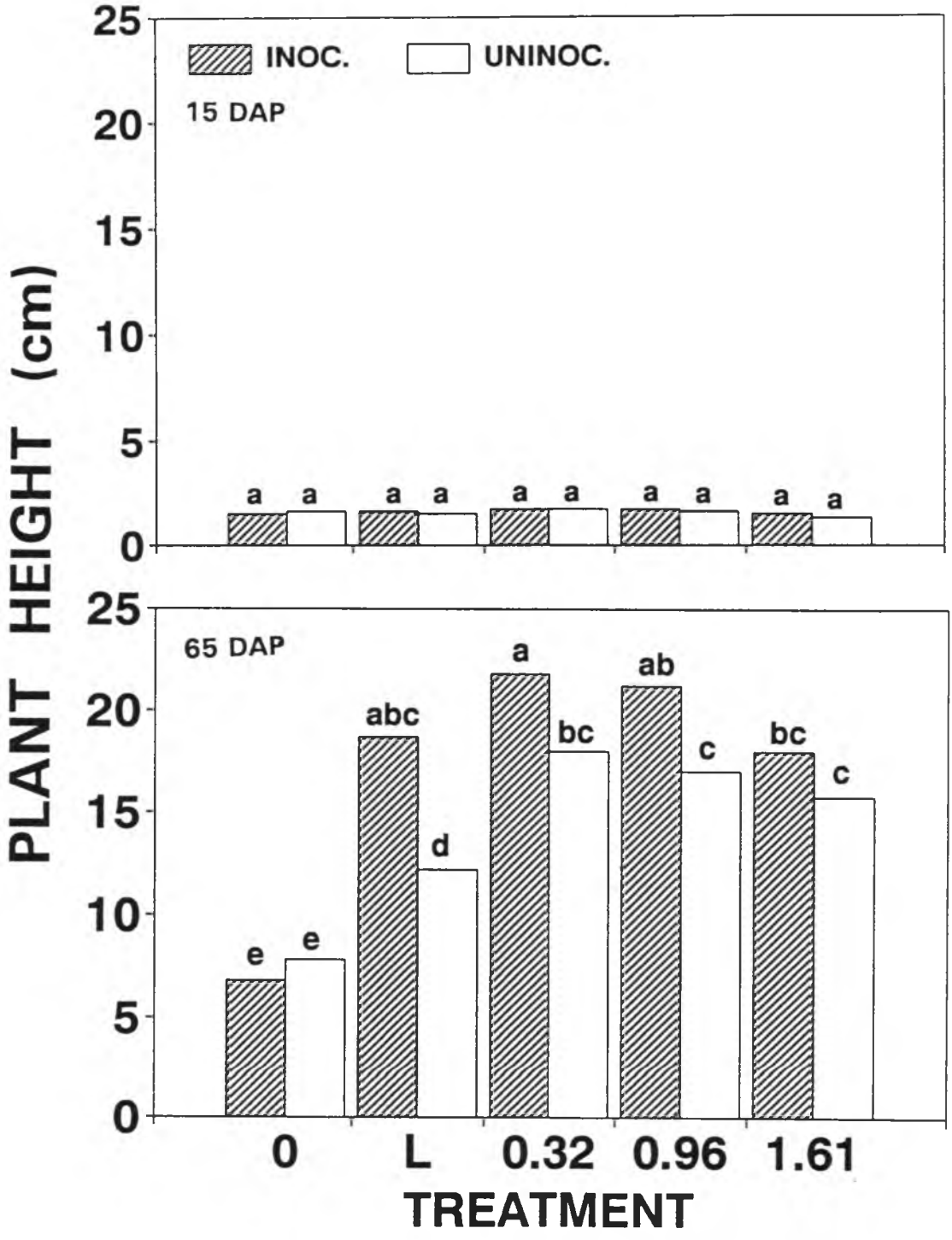

Fig. 4.11. The influence of VAM inoculation and lime or gypsum on plant height of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

DAP, the influence of lime or gypsum was evident in both leucaena and acacia. Plant height of leucaena grown in soil with 0.02 mg P L^{-1} and inoculated with *G. aggregatum* was higher than that of leucaena grown in uninoculated soil except in the soil amended with the highest level of gypsum. At higher soil P level, the tallest leucaena was observed in the limed soil and inoculated with *G. aggregatum*. Gypsum applied at levels higher than 0.32 g Ca kg^{-1} soil generally reduced plant height of leucaena and acacia.

Chemical Composition of Plants

In soil with target P level of 0.02 mg L^{-1}, VAM inoculation significantly increased tissue Cu content of leucaena if soil was limed or amended with the lowest quantity of gypsum (Fig. 4.12). Higher gypsum levels decreased the difference in tissue Cu between inoculated and uninoculated treatments and became insignificant. When soil P was raised to 0.8 mg P L^{-1}, a significant increase of Cu due to inoculation of *G. aggregatum* was observed only in the limed soil.

Total Cu content in acacia was increased by lime or gypsum amendment, and further increase was obtained when soil was inoculated with *G. aggregatum* (Fig. 4.13). The highest Cu content in acacia was attained in inoculated soil and amended with the lowest quantity of gypsum.

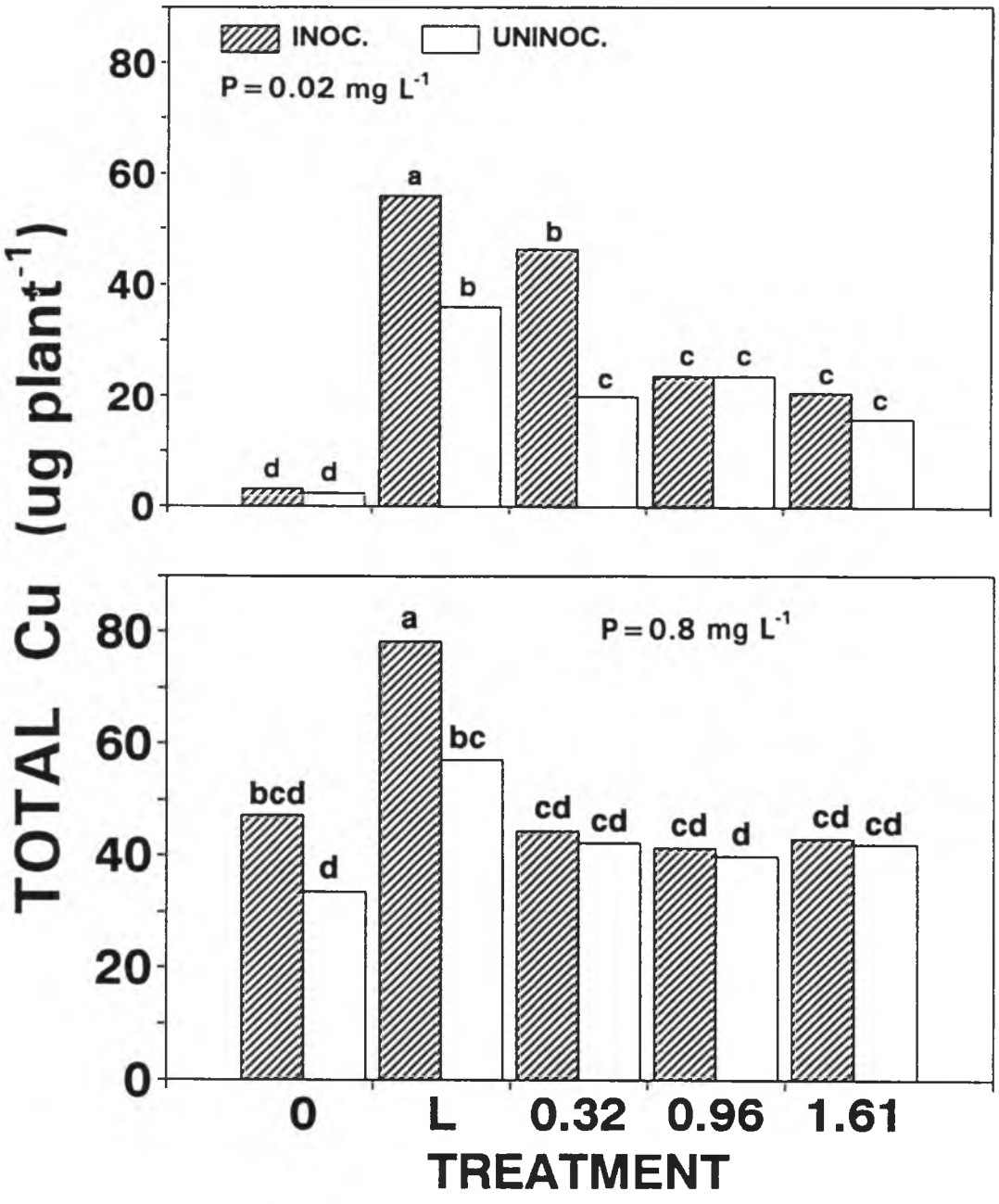

Fig. 4.12. The influence of VAM inoculation, lime or gypsum and P concentration on total shoot Cu content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

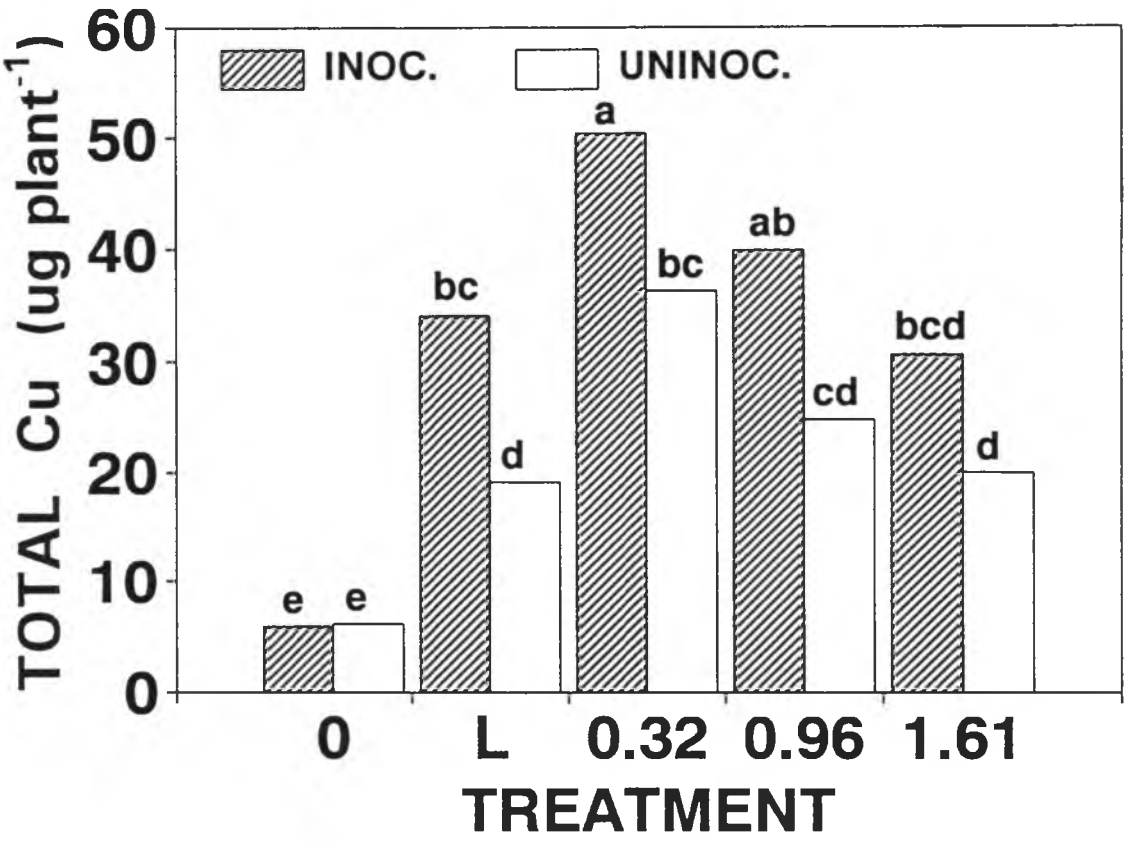

Fig. 4.13. The influence of VAM inoculation and lime or gypsum on total shoot Cu content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

Addition of lime or gypsum increased shoot Zn content of leucaena at the low P level, but only in the limed soil did mycorrhizal inoculation result in higher Zn uptake by leucaena (Fig. 4.14). This figure depicts that shoot Zn status of leucaena grown in the inoculated and in the uninoculated soil with higher P level were similar, except in the limed soil in which mycorrhizal inoculation caused higher Zn uptake by leucaena.

Gypsum was superior to lime in increasing shoot Zn content of acacia (Fig. 4.15). Moreover, the gypsum amendment, not the lime amendment, was accompanied by enhanced Zn uptake by acacia if soil was inoculated. Amendment of gypsum at 1.61 g Ca kg^{-1} reduced shoot Zn content if acacia was grown in uninoculated soil.

Mycorrhizal inoculation did not reduce shoot Mn content of leucaena and acacia, respectively (Fig. 4.16 and 4.17) even though VAM activity in inoculated soil was higher than in uninoculated one (see fig. 4.3-4.5). Lime significantly reduced shoot Mn content of leucaena and acacia. The highest shoot Mn content of leucaena at lower soil P level was observed at the highest gypsum concentration; at the higher soil P level, shoot Mn content at the highest gypsum concentration was similar to that at gypsum concentration of 0.96 g Ca kg^{-1}. High shoot Mn content in acacia was observed at the untreated soil, in the soil treated with the two highest increments of gypsum.

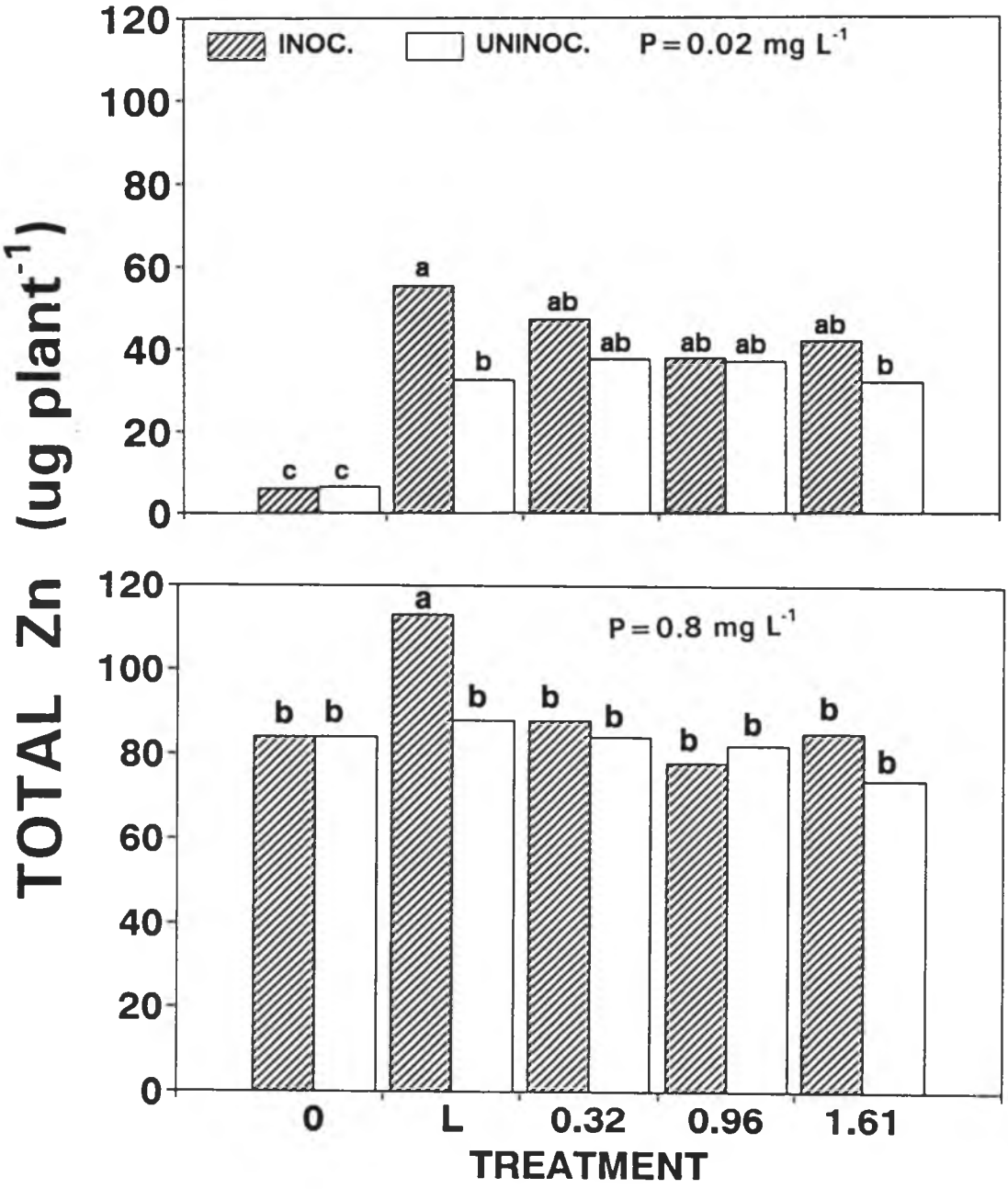

Fig. 4.14. The influence of VAM inoculation, lime or gypsum and P concentration on total shoot Zn content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

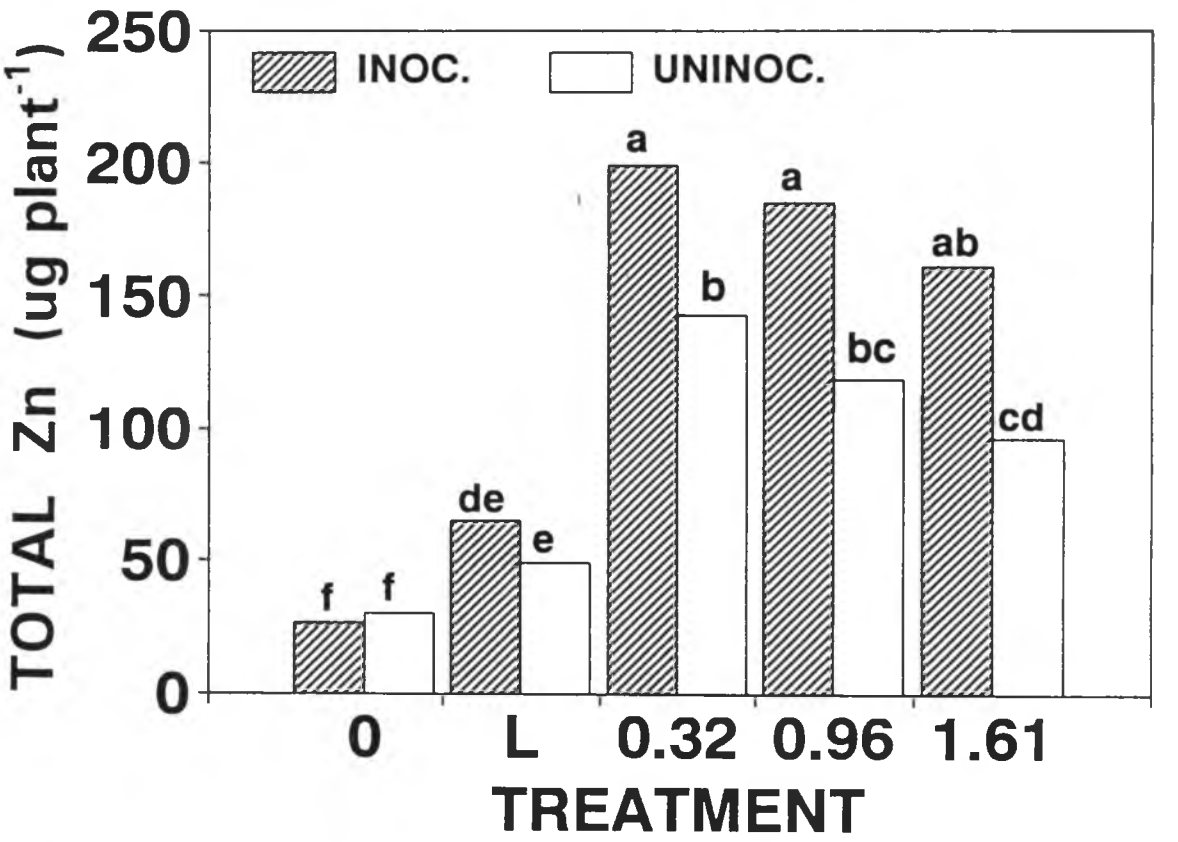

Fig. 4.15. The influence of VAM inoculation and lime or gypsum on total shoot Zn content of acacia. Histograms with the same letters are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

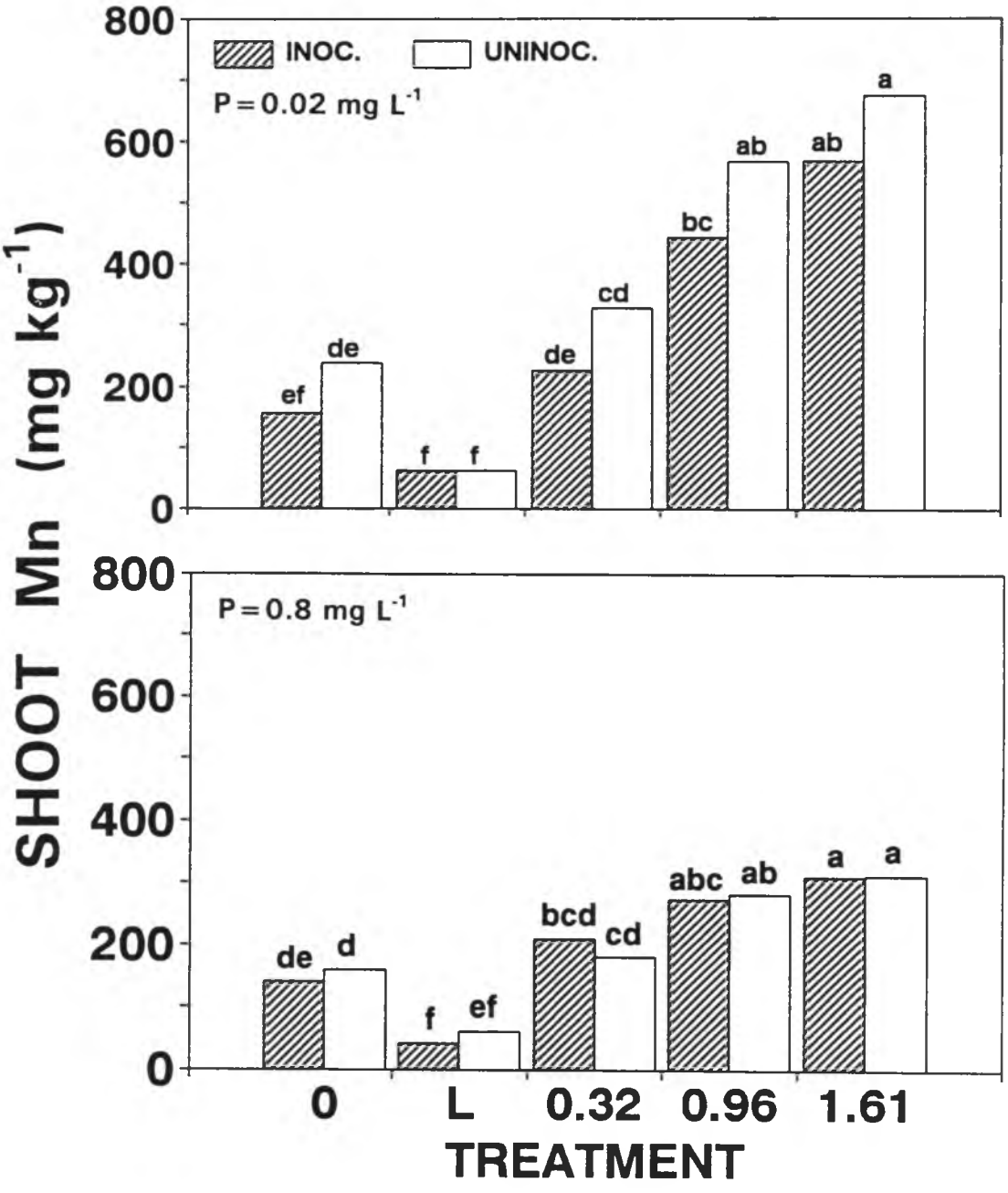

Fig. 4.16. The influence of VAM inoculation, lime or gypsum and P concentration on shoot Mn content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

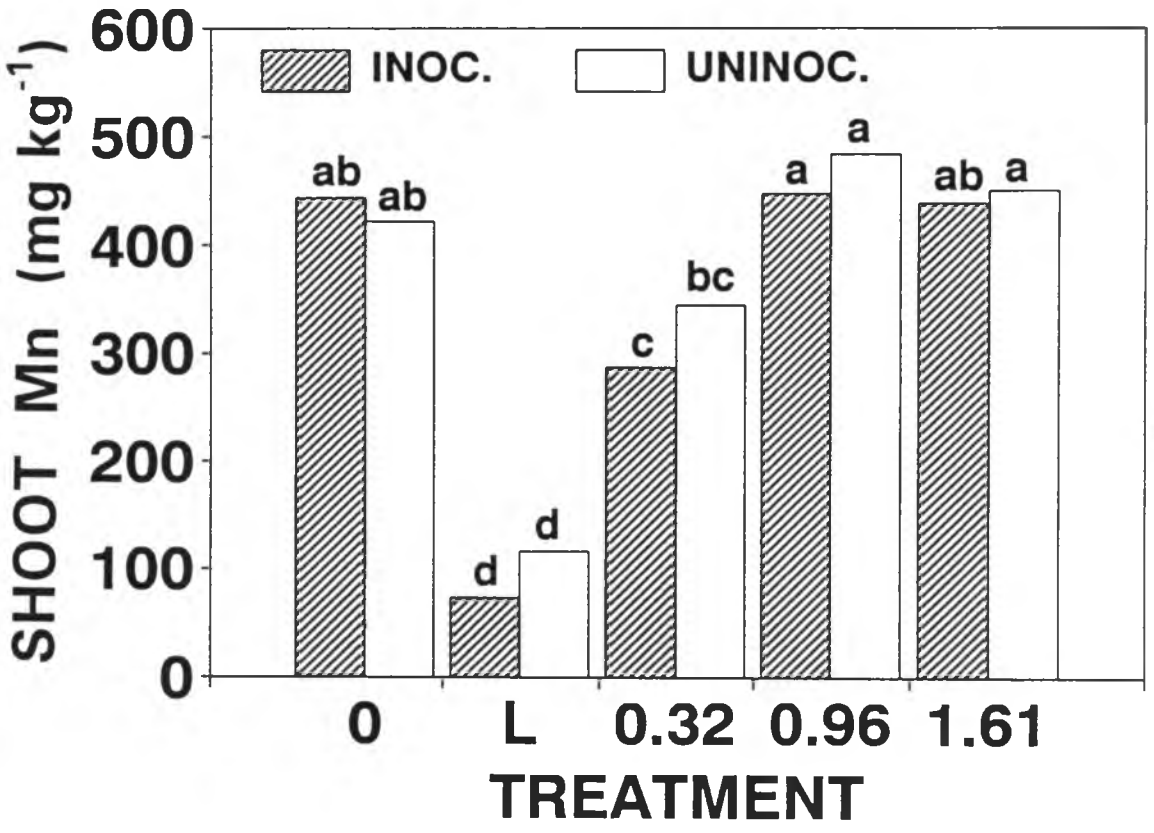

Fig. 4.17. The influence of VAM inoculation and lime or gypsum on shoot Mn content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

In general, shoot Ca was increased when lime or gypsum was added (Fig. 4.18 and 4.19). The highest shoot Ca was observed in leucaena or acacia grown in soil amended with lime. At P level of 0.02 mg L^{-1}, mycorrhizal inoculation stimulated Ca uptake by leucaena if the soil was amended with lime or the lowest amount of gypsum. there was no effect of mycorrhizal inoculation on Ca uptake by leucaena at higher soil P level. Mycorrhizal inoculation stimulated Ca uptake by acacia at the limed or gypsum-treated soil.

Amendment with lime or gypsum enhanced total shoot Mg content of leucaena grown in soil with 0.02 mg P L^{-1} but not that grown in soil with higher P (Fig. 4.20). At lower soil P level, a significant increase due to mycorrhizal inoculation was observed in the limed soil and in the soil treated with the lowest amount of gypsum. There was no effect mycorrhizal inoculation on Mg uptake by leucaena at the higher soil P level. Total shoot Mg content of acacia was augmented by an addition of lime or gypsum (0.32 g Ca kg^{-1}) (Fig.4.21). However, a reduction in shoot Mg was observed if gypsum exceeded 0.32 g Ca kg^{-1}. Mycorrhizal inoculation enhanced Mg uptake by acacia grown in the limed or gypsum-amended soil.

Total shoot P content of leucaena increased significantly when soil was amended with lime and inoculated with *G. aggregatum* irrespective of soil P concentration (Fig. 4.22). Gypsum addition at 0.32 g Ca kg^{-1} also enhanced

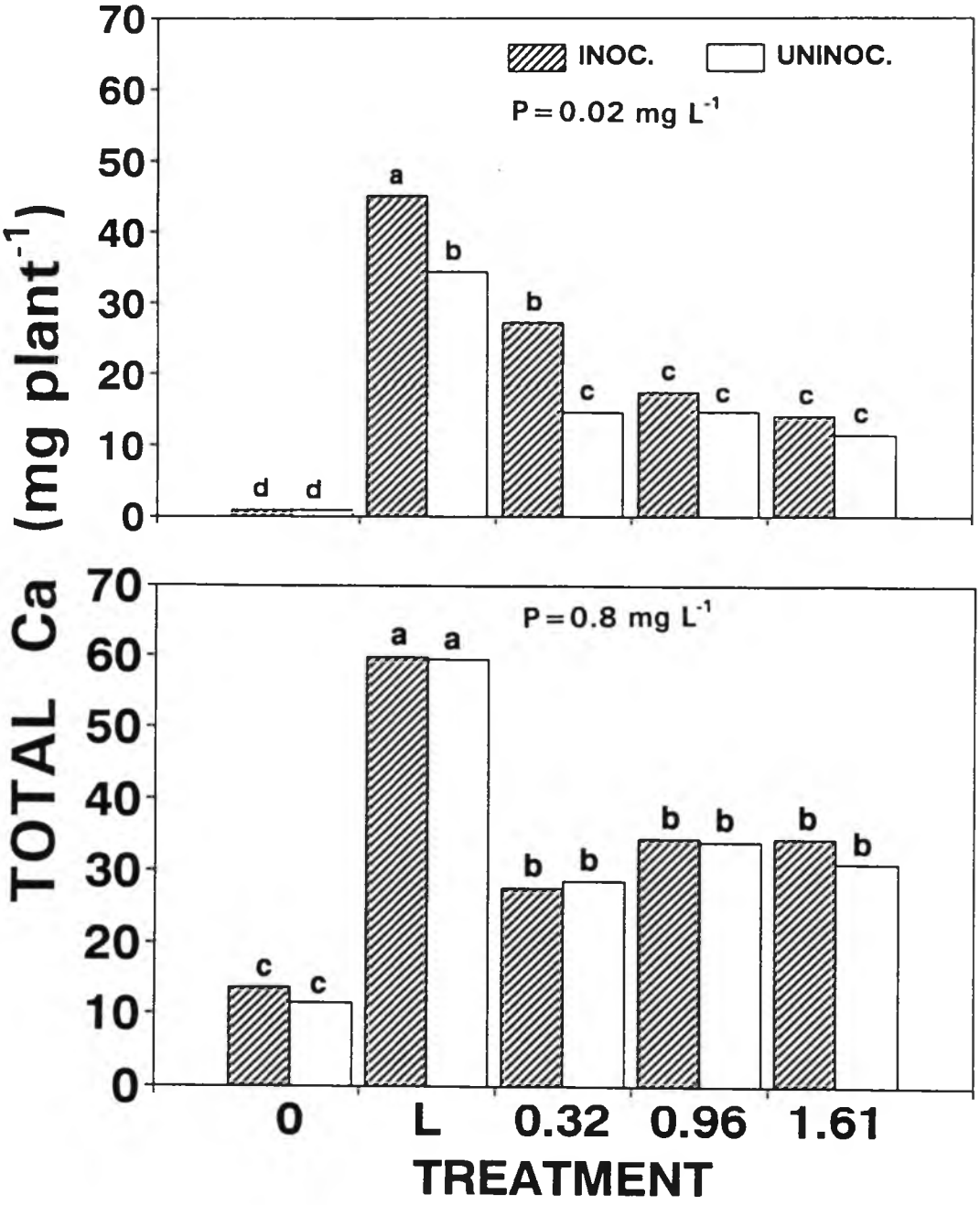

Fig. 4.18. The influence of VAM inoculation, lime or gypsum and P concentration on total shoot Ca content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

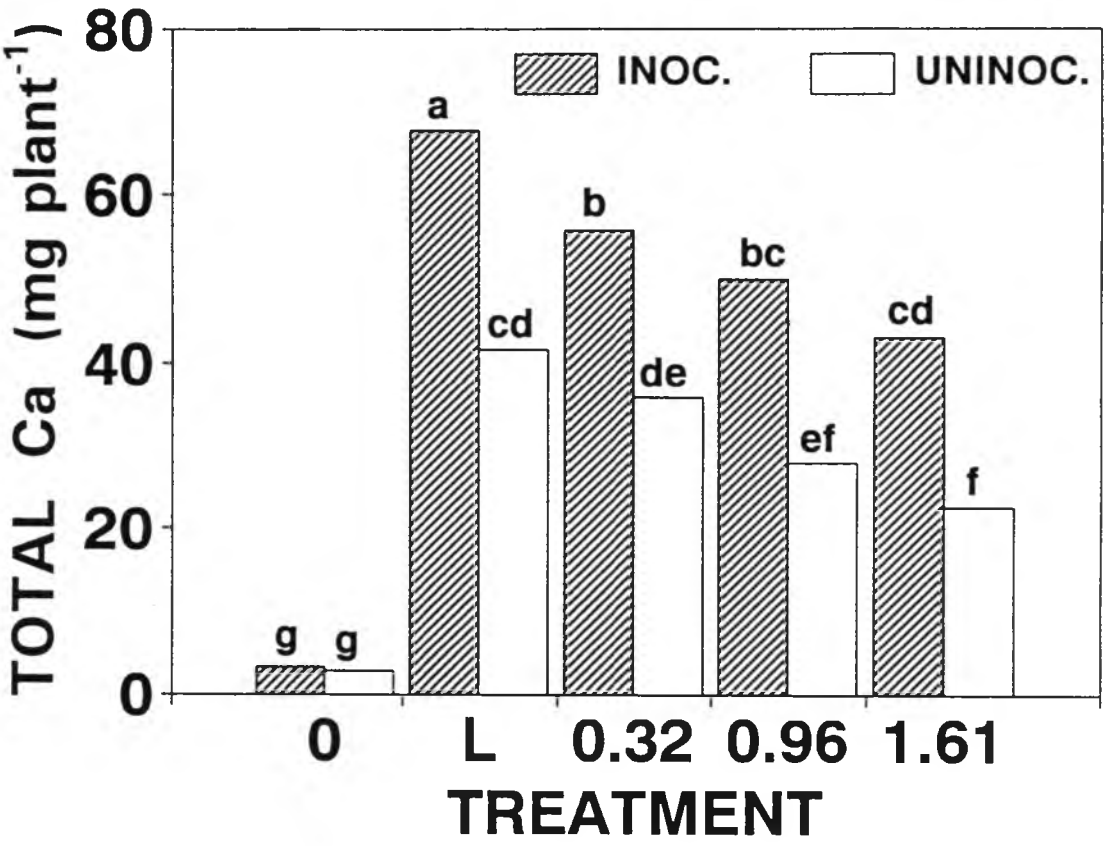

Fig. 4.19. The influence of VAM inoculation and lime or gypsum on total shoot Ca content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

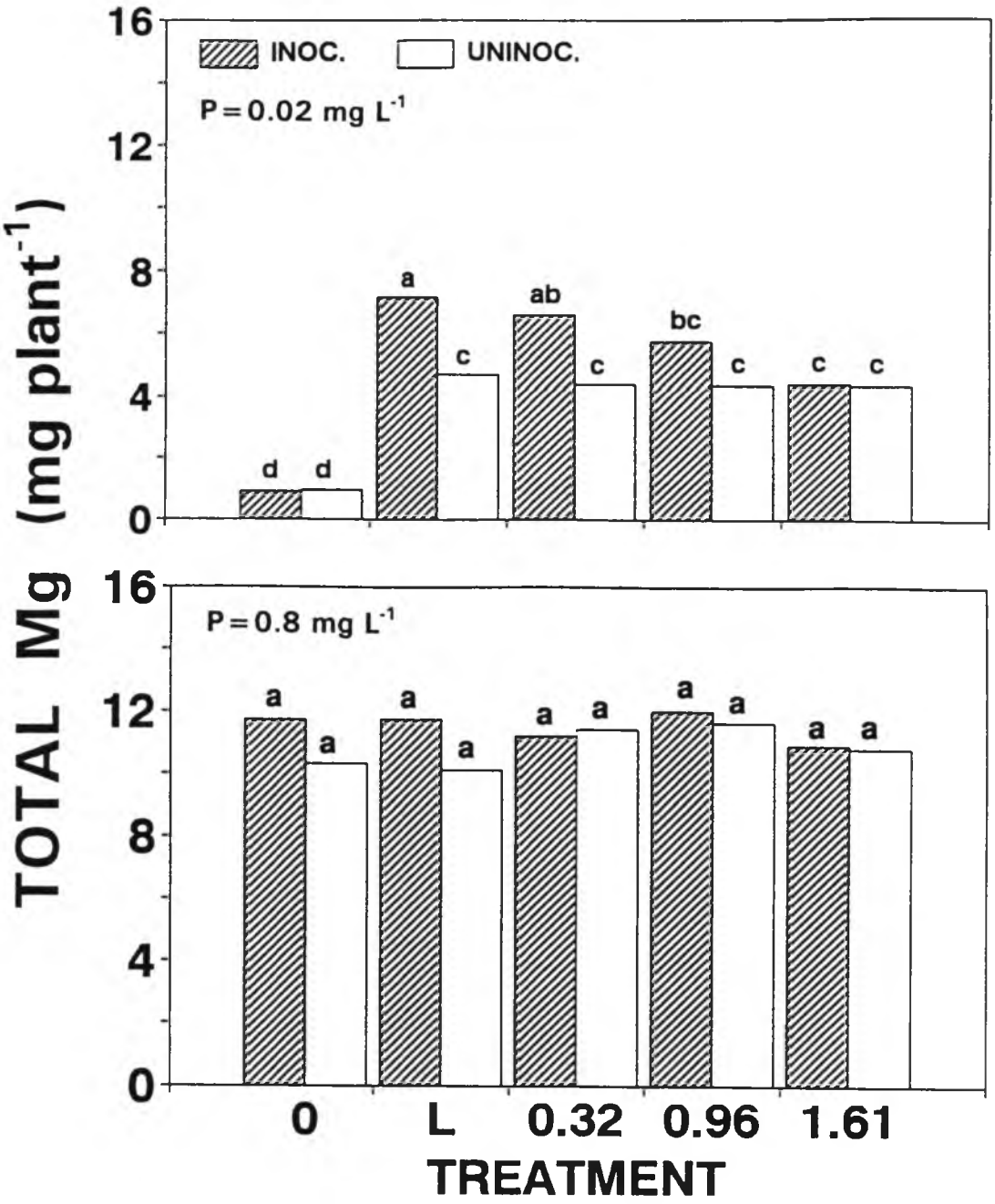

Fig. 4.20. The influence of VAM inoculation, lime or gypsum and P concentration on total shoot Mg content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

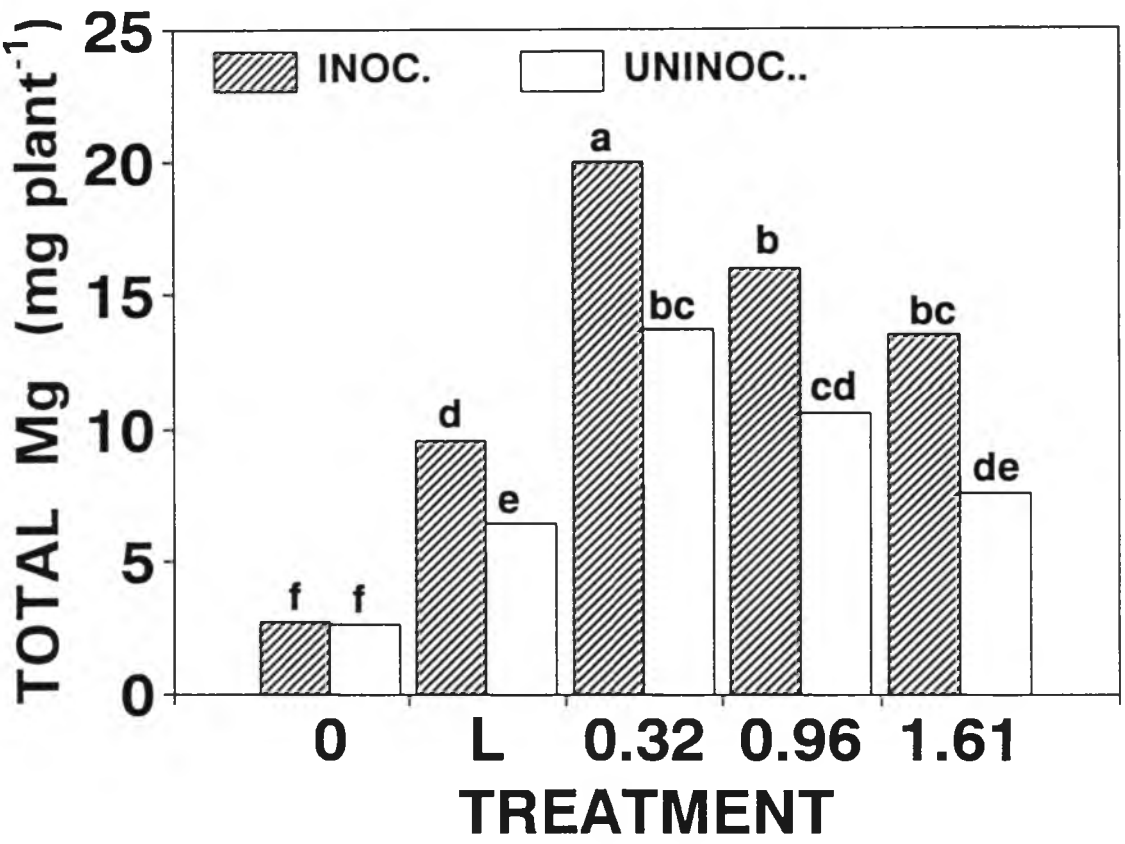

Fig. 4.21. The influence of VAM inoculation and lime or gypsum on total shoot Mg content of acacia. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

mycorrhizal effectiveness in terms of total P of leucaena at a soil P level of 0.02 mg L^{-1} but not at 0.8 mg L^{-1}. Further addition of gypsum diminished the effectiveness of mycorrhizal inoculation. Amendment with gypsum at the higher soil P level led to similar shoot P content of leucaena in inoculated and uninoculated soil. Indeed, addition of gypsum to soil with high P produced pinnules with similar P status in leucaena irrespective of VAM inoculation (Fig. 4.4).

The shoot P content of acacia grown in soil amended with the lowest amount of gypsum and inoculated with G. aggregatum was not different from that of acacia grown in the limed and inoculated soil (Fig. 4.23). Mycorrhizal inoculation enhanced total P uptake by acacia grown in the limed or gypsum-amended soil. Nevertheless, at gypsum amendment exceeding 0.32 g Ca kg^{-1} total P uptake was reduced regardless of VAM inoculation.

DISCUSSION

Soil Chemical Properties Before Planting and After Harvest

Hydroxyl ions are released following lime addition resulting in an increase of soil pH. Besides hydroxyl ion, calcium is also released and this causes an increase of soil Ca.

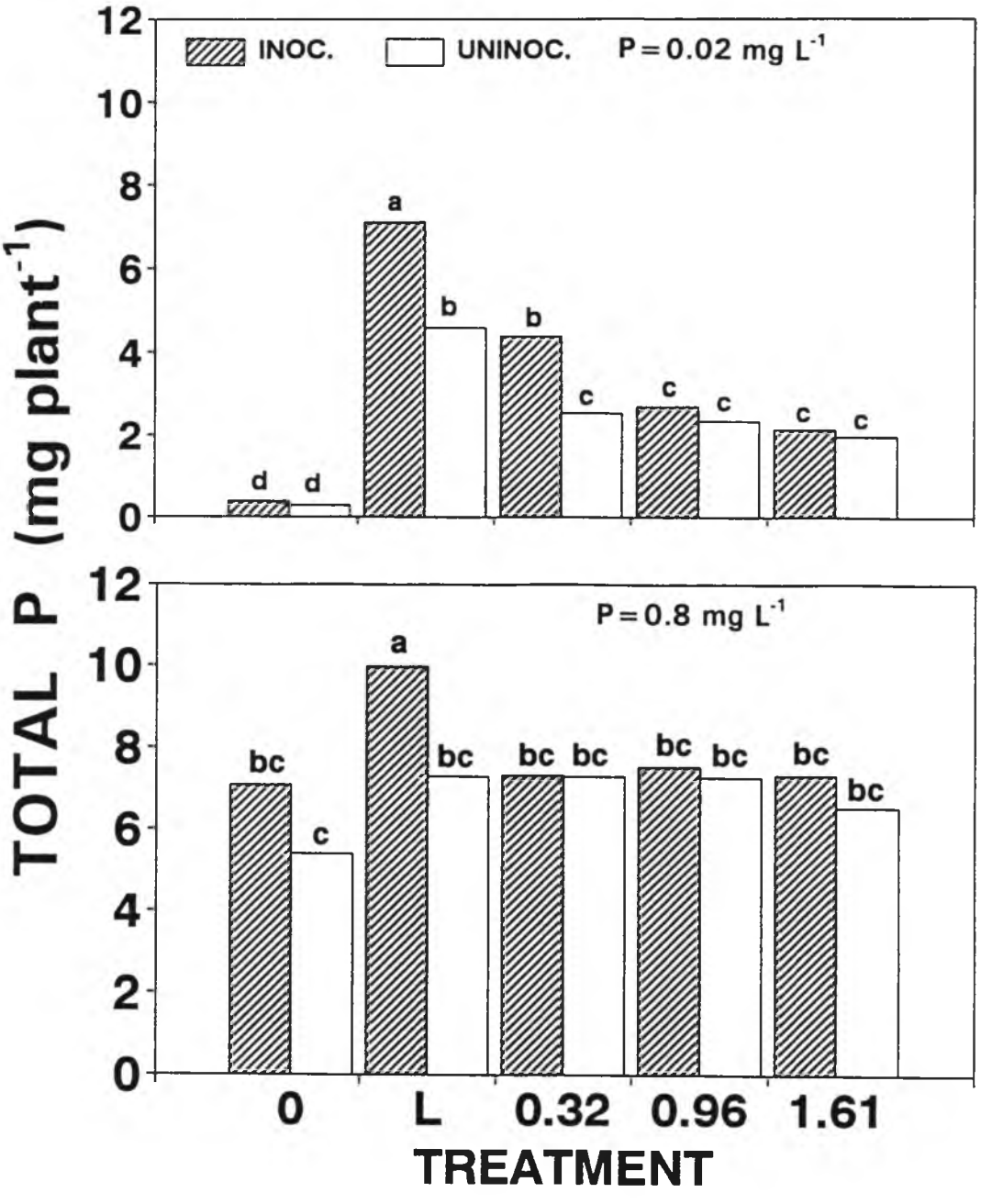

Fig. 4.22. The influence of VAM inoculation, lime or gypsum and P concentration on total shoot P content of leucaena. Histograms with the same letter are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum $\{Ca(SO)_4\}$; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

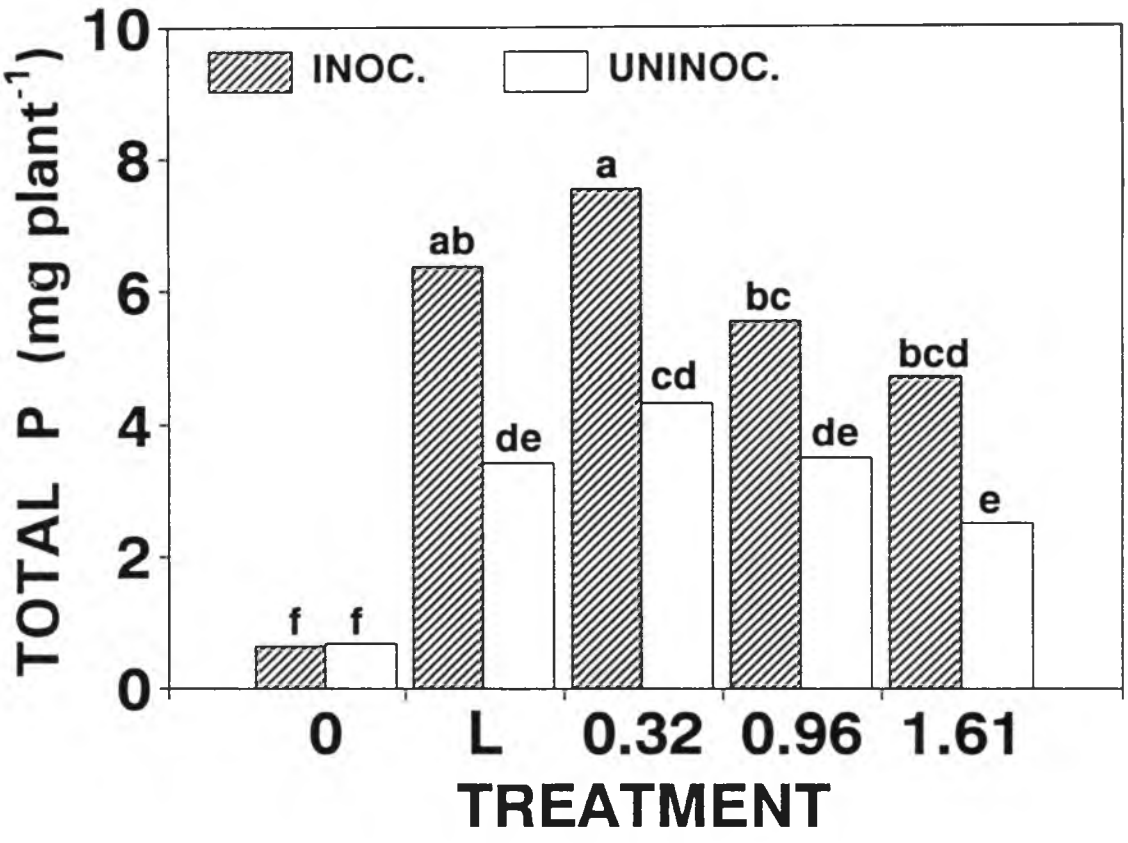

Fig. 4.23. The influence of VAM inoculation and lime or gypsum on total shoot P content of acacia. Histograms with the same letters are not significantly different at the 5% probability level by the L.S.D test. 0, 0.32, 0.96, 1.61 represent grams of Ca added in the form of gypsum {$Ca(SO)_4$}; L= lime, $Ca(OH)_2$ (1.61 g Ca kg^{-1}).

A decrease of soil pH observed in the current study after gypsum amendment at quantities higher than 0.32 g Ca kg^{-1} could be due to sulfur which has a low isoelectric point (Uehara, personal communication). Since soil pH was decreased with these treatments, Mn availability was increased. The pH at these treatments is considered to be acid, and under such conditions unavailable forms of manganese (MnO_2) are converted to available manganese (Mn^{+2}).

Mn reduction after lime or the lowest amount of gypsum amendment is due to an increase in soil pH. Higher soil pH has been reported to reduce Mn availability (Fox *et al.*, 1991). At higher pH Mn is precipitated as $Mn(OH)_2$ (Ritchie, 1989).

P added to the soil might produce excess phosphate. Some of the phosphate ion will replace hydroxyl ions adsorbed by the soil leading to increase in the concentration of hydroxyl ions in soil solution. Hence, this process may explain the increase in soil pH at the high P level. This is likely to happen in the soil used in the current study because the soil is an Oxisol with pH-dependent charge (Uehara and Gillman, 1981). Consequently, solubility of Mn in soil amended with the higher P concentration was reduced (see Table 4.3). This was evidenced by the absence of Mn toxicity symptom in leucaena grown on the soil.

Increased Ca content observed in soil solution after gypsum or lime addition is apparently due to Ca coming into solution. Excess Ca from lime or gypsum amendment may replace Mg adsorbed on soil colloids and thus may cause an increase in the concentration of Mg in the soil solution.

After harvest, reduction in soil pH was observed in the soil with target P level of 0.02 mg L^{-1}. In the course of the experiment CO_2 was produced by soil microorganisms whose proliferation was enhanced by organic carbon released by roots of plants (Whipps and Lynch, 1986); as microorganisms decompose this organic matter, more CO_2 is evolved. The more CO_2 released the higher the concentration of hydrogen ions produced; consequently, soil pH is reduced. Since charge of the soil is pH-dependent, the soil might become more positively charged with decrease in pH. As a result, cations like Ca and Mg are repelled and released into the soil solution. However, in the soil with the higher P level, soil Mn, Ca, Mg were lower after harvest than before planting. Hence increase in soil pH due to P amendment is likely to be accompanied by the reduction in the concentration of soil Mn, Ca, and Mg. In higher pH, available Mn is precipitated as $Mn(OH)_2$ (Ritchie, 1989). A reaction of Mn with an excess of phosphate ion might also have contributed to the reduction of Mn (Norvell, 1988). Since the soil might become negatively charged under this condition, more Ca and Mg were adsorbed which resulted in

the reduction of their concentrations in the soil solution.

Vesicular-Arbuscular Mycorrhizal (VAM) Colonization

Soil amended with lime seems to be conducive to the development of *G. aggregatum*. It might be due to higher soil pH, sufficient Ca or non toxic levels of Mn (see Tables 4.1, 4.2, and 4.3). However, soil pH might not be one of the factors in this soil restricting VAM development, because at pH 5.1 (lowest level of gypsum), VAM colonization was as high as at pH 6.0 (see Fig. 4.1). Moreover, soil treated with gypsum higher than 0.32 g Ca kg^{-1} had pH lower than the untreated soil (Tables 4.1-4.3) yet VAM colonization was higher than in the untreated soil. Soil amended with the lowest amount of gypsum contained a higher quantity of Ca than the untreated soil, suggesting that sufficient Ca should be present in soil for the development of VAM fungi. The nutrient film technique used by Elmes *et al.*(1980) revealed that VAM colonization in roots of corn at 75 mg Ca L^{-1} was better than that at 15 or 150 mg Ca L^{-1}. Soedarjo and Habte (in Press) compared the effect of fresh organic matter and lime and noted a higher level of VAM colonization when an acid Ultisol was treated with lime than with fresh organic matter, even though Al toxicity was effectively nullified in both cases. The better VAM development observed in the limed soil, they concluded, was due to a better Ca

supply. Besides, in soil amended with lime or with the lowest amount of gypsum, Mn was lower than that in the untreated soil or in the soil treated with higher gypsum concentrations. The Mn level in soil treated with higher amounts of gypsum might be toxic to VAM development. Mn was found to be toxic to VAM colonization (Wang *et al.* 1985). Therefore, either low Ca, toxic Mn or both have limited VAM colonization in some acid soils in the current study.

Development of Vesicular-Arbuscular Mycorrhizal (VAM) Activity

UNDERLINE

Time required to reach the peak of mycorrhizal activity of leucaena in the current study was 20-25 days longer than those observed by Habte and Manjunath (1987), Manjunath and Habte (1990), and Habte and Manjunath, (1991). Peak of mycorrhizal activity in acacia was not attained at the termination of the experiment. After 45 DAP, pinnule P content was not determined because subleaflets were not formed. Growth of leucaena and acacia during the first 40 DAP was slow (visual observation). As a result, photosynthate produced might be inadequate for VAM fungi activity. Since VAM fungi rely on carbon supply from the host plant (Thompson, 1990), their activity might have been delayed accordingly.

The greater VAM activity in soil amended with lime or that amended with the lowest amount of gypsum might be related to non-toxic levels of Mn or greater Ca supply than in the untreated soil or in soil treated with higher levels of gypsum. Soil pH as low as 5.0 did not limit *G. aggregatum* activity since soil treated with the lowest amount of gypsum (pH of 5.1) showed greater VAM activity if the soil was inoculated with *G. aggregatum* than if not inoculated. Besides the greater Ca status of the soil treated with the lowest quantity of gypsum compared to untreated soil, Mn concentration was lower. Thus, it is likely that the insufficiency of soil Ca or the high level of Mn seemed to restrict VAM activity in the acid soil studied.

Shoot Dry Weight

Compared to the soil amended with the lowest amount of gypsum, the limed soil was characterized by higher pH and higher Ca content. Leucaena has been reported to be sensitive to acid soils (Hutton, 1981; Olvera *et al.*, 1982; Balasundaran *et al.*, 1988; Halinda, 1988). Thus better plant growth observed in the lime treatment could be due in part to higher soil pH and higher Ca content as well.

At soil P concentration of 0.02 mg L^{-1}, VAM colonization of leucaena grown in the limed soil and in the soil amended with the lowest gypsum level were similar (Fig. 4.1).

However, they did not have comparable shoot dry weight. It is likely that the better plant growth in the limed inoculated soil was due to greater abundance of extramatrical hyphae. Graham *et al.* (1982) noted differences in growth enhancement by different endophytes due to differences in the development of external hyphae, even though there were no differences in the extent of root colonization.

Leucaena grown in the untreated soil did not show Mn toxicity at low P level although it was stunted. Compared to the limed soil, the untreated soil had a lower pH and a much lower Ca content. The pH of the untreated soil was higher than that of the soil treated with gypsum. However, the pH difference between the untreated soil and the gypsum treated soil might not result in different effect on plant growth. Soil treated with gypsum, nevertheless, was characterized by higher Ca content compared to the untreated soil. Untreated soil and soil treated with gypsum for all practical purpose were acidic. Therefore, besides unsuitable pH, poor growth of leucaena in the untreated soil could be also due to low Ca content.

A reduction of shoot dry weight of leucaena and acacia after gypsum amendment at levels higher than 0.32 g Ca kg^{-1} might be related to Mn toxicity. Elmes *et al.* (1980) and Siqueira (1983) noted an adverse effect of Ca when applied in excess. Therefore, toxicity of Mn or an excess of Ca or

both might have been responsible for the reduction of shoot dry weight.

Yield increases of acacia observed due to the addition of lime and the lowest amount of gypsum in inoculated soil were 594% and 919%, over those of the untreated soil and inoculated with same endophyte, respectively. The Mn content of the soil amended with the lowest amount of gypsum was similar to that of the limed soil but had lower pH, and lower Ca content in the soil solution (Tables 4.1 and 4.4). Ca was found to be beneficial to VAM fungi but too much of it can be detrimental (Elmes *et al.* 1980 and Siqueira, 1983). On the other hand, acacia was reported to be tolerant to acid soils (Glover and Heuveldop, 1985; Halinda, 1988). Thus, besides appropriate soil pH, sufficient soil Ca is probably required for optimum acacia-mycorrhizal symbiosis.

Mn toxicity symptoms were observed on acacia grown in the untreated soil which indicates that toxic levels of Mn in this soil occurred and this was related to low pH (see Table 4.4). Lower Ca in the untreated soil than in soil amended with lime or gypsum was also observed. The hydrogen ion does not seem to be responsible for the poor growth of acacia in the untreated soil because the legume treated with gypsum grew better in the soil amended with the first increment of gypsum even though the pH of this soil was comparable to that of the untreated soil (Fig. 4.23). Thus poor growth of acacia in the untreated soil is most likely

related to Mn toxicity and /or insufficient Ca. Insufficient Ca and/or Mn toxicity could be the cause of the absence of mycorrhizal formation in untreated soil (see Figs. 4.1 and 4.2).

Hydrogen ion itself did not seem to adversely influence mycorrhizal effectiveness in terms of shoot dry weight. This is evidenced by the fact that shoot dry weight of acacia grown in inoculated soil amended with gypsum at concentration of 0.96 g Ca kg^{-1} was higher than that of acacia grown in the untreated soil even though pH of the former soil was much lower than pH of the latter soil (Fig. 4.7 and Table 4.1). Mn toxicity seems to be the component of acid soil toxicity involved in reduction of mycorrhizal effectiveness. Mn concentration in soil amended with gypsum at quantity of 0.96 g Ca kg^{-1} and 1.61 g Ca kg^{-1} was higher than that of soil treated with the amount of gypsum resulting in lower plant growth. Toxic effects of metals such as Al, and Mn were reported by Siqueira *et al.*, (1984) and Wang *et al.* (1985).

The soil used in the current study was not sterilized and thus indigenous mycorrhizal fungi were present in it. The soil was collected from the Wahiawa series at a depth of 7.5 to 15 cm. Habte (1989) recovered more than 10 infective propagules from the same soil and with this amount of infective propagules mycorrhizal activity was detected. However, the present study revealed that infectivity and

effectivity of indigenous endophyte were inferior to those of introduced VAM fungi. The Mycorrhizal inoculum used probably contained higher infective propagules and was more effective than the indigenous VAM. Lower effectivity of indigenous VAM from the Wahiawa soil was documented elsewhere (Aziz and Habte, 1990; Habte and Aziz, 1991).

Root Dry Weight

Phosphorus is required for root growth (Salisbury and Ross, 1985). Thus, higher root dry weight of leucaena in inoculated soil than in uninoculated soil amended with lime or the lowest amount of gypsum is due to higher internal P. Aziz and Habte (1987) and Habte and Aziz (1991) noted higher leaf P status in mycorrhizal plants than in non-mycorrhizal plants which was accompanied by higher root dry weight. By the same token, high internal P of acacia grown in the inoculated soil amended with the lowest amount of gypsum accounted for the highest root dry weight. The failure of the introduced VAM fungus to function better than the indigenous endophytes in leucaena grown in soil amended with the higher levels of gypsum (see Figs. 4.3 and 4.22) probably explains the failure of the introduced VAM fungus to stimulate root dry weight production. When soil P was increased to 0.8 mg L^{-1}, only in the limed soil did mycorrhizal inoculation enhance root dry weight production

of leucaena. Root dry weight is in good agreement with mycorrhizal activity measured in terms of pinnule P content and total nutrient uptake, especially P. Thus this result support the previous findings by Aziz and Habte (1987), Habte and Manjunath (1987), Aziz and Habte (1989b), Habte and Aziz (1991) who reported that high mycorrhizal activity was accompanied by higher root dry weight.

Plant Height

Leucaena grew better at the higher P level than at the lower soil P level and as a result, the plants were taller. At this soil P level, the tallest leucaena plants were observed in the limed soil inoculated with *G. aggregatum*. Better growth of leucaena in this soil could be explained by improved nutrient supply (Cu, Zn, Ca, Mg, and P). For the same reason, better growth of acacia in the inoculated soil amended with the lowest amount of gypsum could be explained by the better nutrient status.

Chemical Composition of Plants

A previous study (Manjunath and Habte, 1988) has found higher uptake of immobile micronutrients (Cu and Zn) by mycorrhizal plants than by non-mycorrhizal plants. The present study agrees with this finding. However, in acid

tropical soils such as Oxisols, proper management regarding soil pH, amendment of some nutrients,i.e., Ca needs to be taken into account to maximize the role of mycorrhizal colonization in increasing immobile micronutrient uptake. This management depends on plant species. The current study suggests that Ca be added to an acid soil. It is sensible to add Ca since the soil used in the present study contain low Ca (see Table 4.1) and the beneficial effect of Ca on VAM symbiosis was reported by Elmes *et al*. (1980), Soedarjo and Habte (in press). Based on the current study, addition of 0.32 g Ca kg^{-1} soil is considered to be sufficient for acacia-VAM fungi symbiosis, but soil needs to be limed to about 6.0 for optimum leucaena-mycorrhizal symbiosis.

Similar shoot Mn content of leucaena and acacia (Figs. 4.16, and 4.17) in the inoculated and in the uninoculated soil regardless of soil P concentration and lime or gypsum amendment indicates that mycorrhizal fungi do not play a role in alleviating Mn toxicity. High Mn concentration in the soil solution was accompanied by high Mn content in plant tissue suggesting a good agreement between soil Mn concentration and shoot Mn content. Vega *et al*. (1992) found that soil-solution Mn was a good indicator of Mn phytotoxicity. Lower shoot Mn of leucaena in soil with the higher P rate was related to low availability of Mn (Fig. 4.16).

Calcium was measured as total Ca uptake per plant. The decrease of Ca content per plant after addition of gypsum at the rate higher than 0.32 g Ca kg^{-1} was related to the reduction of plant growth even though Ca in the soil solution at these treatments were higher than Ca content in soil treated with lime or the lowest amount of gypsum (see Tables 4.1-4.3).

A reduction in mycorrhizal enhancement of P uptake after gypsum addition at quantities higher than 0.32 g Ca kg^{-1} might be related to Mn toxicity or detrimental effect of high Ca (see Tables 4.1 and 4.2). An increase of P uptake due to mycorrhizal colonization has been documented elsewhere (Aziz and Habte, 1987; Manjunath and Habte, 1988; Aziz and Habte, 1989a; Manjunath and Habte, 1989; Aziz and Habte, 1990; Habte and Turk, 1991; Manjunath and Habte, 1991). A positive response of leucaena to mycorrhizal inoculation in limed soil with higher P probably reflects the very highly mycorrhizal dependency of leucaena. Previous investigators (Habte and Manjunath, 1991; Habte and Manjunath, 1987) have observed leucaena responses to mycorrhizal inoculation in soil with P levels sufficient for non-mycorrhizal host growth of most plants.

Addition of gypsum to soil at high P reduced differences in P status of pinnules of leucaena irrespective of VAM inoculation (Fig. 4.4). Thus, the inability of *G. aggregatum* to enhance P uptake of leucaena above that

attained in the uninoculated soil is due to the inhibitory effect of addition of gypsum in excess of 0.32 g Ca kg^{-1} soil. This inhibitory effect might be due to Mn toxicity, excess Ca or both (see Tables 4.1-4.2). The superiority of the lime treatment over the other treatments probably was due to a more suitable pH. After harvest, pH of the limed soil was 6.1 which was higher than the pH of the untreated soil, or that of soil amended with gypsum (Table 4.2).

CONCLUSIONS

The results of the current study suggest that soil pH did not directly influence mycorrhizal effectiveness. Beneficial effects of Ca amendment on VAM effectiveness could not be separated from those of low concentration of soil Mn. Sufficient Ca in the soil solution required to improve mycorrhizal effectiveness might be different from one plant species to another. Mn was most likely to restrict VAM effectiveness; therefore, soil pH must be raised beyond the pH necessary to alleviate Mn toxicity if acid sensitive host like *Leucaena leucocephala* is to be grown. *Acacia mangium*, on the other hand, seems to be tolerant to acid soil which is low in Mn, therefore soil pH needs to be increased to a level necessary to alleviate Mn toxicity in order to get optimum benefits from mycorrhizal inoculation.

However, in the first experiment, I was not able to differentiate the effect of soil pH on VAM symbiosis from that of Mn because at low pH, Mn availability was high. In the second experiment, Ca^{+2} effects on VAM symbiosis could not be separated from that of Mn^{+2} and H^{+} because untreated soil had high Mn^{+2} and H^{+} ions besides low Ca^{+2} concentration in the soil solution. Therefore, I propose that 2 experiments need to be conducted. Firstly, medium which is inherently low in Mn such as quartz sand could be used to study the effect of soil pH on VAM effectiveness. Secondly, medium which is low in Ca and Mn and which has zero net charge such as the Kapaa soil series at a depth of 60-90 cm could be used to differentiate the effect of Ca^{+2} from that of H^{+} and Mn^{+2}.

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